RNA INTERFERENCE MEDIATED INHIBITION OF XIAP GENE EXPRESSION USING SHORT INTERFERING NUCLEIC ACID (siNA)

This application claims the benefit of U.S. Provisional Application No. 60/493,561 filed August 8, 2003. This application is a continuation-in-part of International Patent Application No. PCT/US04/16390, filed May 24, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/826,966, filed April 16, 2004, which is continuation-in-part of U.S. Patent Application No. 10/757,803, filed January 14, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/720,448, filed November 24, 2003, which is a continuation-in-part of U.S. Patent Application No. 10/693,059, filed October 23, 2003, which is a continuation-in-part of U.S. Patent Application No. 10/444,853, filed May 23, 2003, which is a continuation-in-part of International Patent Application No. PCT/US03/05346, filed February 20, 2003, and a continuation-in-part of International Patent Application No. PCT/US03/05028, filed February 20, 2003, both of which claim the benefit of U.S. Provisional Application No. 60/358,580 filed February 20, 2002, U.S. Provisional Application No. 60/363,124 filed March 11, 2002, U.S. Provisional Application No. 60/386,782 filed June 6, 2002, U.S. Provisional Application No. 60/406,784 filed August 29, 2002, U.S. Provisional Application No. 60/408,378 filed September 5, 2002, U.S. Provisional Application No. 60/409,293 filed September 9, 2002, and U.S. Provisional Application No. 60/440,129 filed January 15, 2003. This application is also a continuation-in-part of International Patent Application No. PCT/US04/13456, filed April 30, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/780,447, filed February 13, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/427,160, filed April 30, 2003, which is a continuation-in-part of International Patent Application No. PCT/US02/15876 filed May 17, 2002, which claims the benefit of U.S. Provisional Application No. 60/362,016, filed March 6, 2002, U.S. Provisional Application No. 60/292,217, filed May 18, 2001, U.S. Provisional Application No. 60/363,883, filed July 20, 2001, and U.S. Provisional Application No. 60/311,865, filed August 13, 2001. This application is also a continuation-in-part of U.S. Patent Application No. 10/727,780 filed December 3, 2003. This application also claims the benefit of U.S. Provisional Application No. 60/543,480, filed February 10, 2004. The instant application claims the benefit of all the listed applications, which are hereby incorporated by reference herein in their entireties, including the drawings.

Field Of The Invention

The present invention relates to compounds, compositions, and methods for the study, diagnosis, and treatment of traits, diseases and conditions that respond to the modulation of X-linked inhibitor of apoptosis protein (XIAP) gene expression and/or activity. The present invention is also directed to compounds, compositions, and methods relating to traits, diseases and conditions that respond to the modulation of expression and/or activity of genes involved in XIAP gene expression pathways or other cellular processes that mediate the maintenance or development of such traits, diseases and conditions. Specifically, the invention relates to small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against XIAP gene expression. Such small nucleic acid molecules are useful, for example, in providing compositions for treatment of traits, diseases and conditions that can respond to modulation of XIAP expression in a subject, such as those associated with the maintenance and/or development of cancer and other proliferative disorders.

Background Of The Invention

The following is a discussion of relevant art pertaining to RNAi. The discussion is provided only for understanding of the invention that follows. The summary is not an admission that any of the work described below is prior art to the claimed invention.

RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Zamore *et al.*, 2000, Cell, 101, 25-33; Fire *et al.*, 1998, *Nature,* 391, 806; Hamilton *et al.*, 1999, *Science,* 286, 950-951; Lin *et al.*, 1999, *Nature,* 402, 128-129; Sharp, 1999, Genes & Dev., 13:139-141; and Strauss, 1999, *Science,* 286, 886). The corresponding process in plants (Heifetz *et al.*, International PCT Publication No. WO 99/61631) is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have

evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response through a mechanism that has yet to be fully characterized. This mechanism appears to be different from other known mechanisms involving double stranded RNA-specific ribonucleases, such as the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L (see for example US Patent Nos. 6,107,094; 5,898,031; Clemens *et al.*, 1997, *J. Interferon & Cytokine Res.*, 17, 503-524; Adah *et al.*, 2001, *Curr. Med. Chem.*, 8, 1189).

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer (Bass, 2000, Cell, 101, 235; Zamore *et al.*, 2000, Cell, 101, 25-33; Hammond *et al.*, 2000, Nature, 404, 293). Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Zamore *et al.*, 2000, Cell, 101, 25-33; Bass, 2000, Cell, 101, 235; Berstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes (Zamore *et al.*, 2000, Cell, 101, 25-33; Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188).

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Bahramian and Zarbl, 1999, *Molecular and Cellular Biology*, 19, 274-283 and Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mammalian systems. Hammond *et al.*, 2000, *Nature*, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir

et al., 2001, *Nature*, 411, 494 and Tuschl *et al.*, International PCT Publication No. WO 01/75164, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in Drosophila embryonic lysates (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877 and Tuschl *et al.*, International PCT Publication No. WO 01/75164) has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21-nucleotide siRNA duplexes are most active when containing 3'-terminal dinucleotide overhangs. Furthermore, complete substitution of one or both siRNA strands with 2'-deoxy (2'-H) or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of the 3'-terminal siRNA overhang nucleotides with 2'-deoxy nucleotides (2'-H) was shown to be tolerated. Single mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end of the guide sequence (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell*, 107, 309).

Studies have shown that replacing the 3'-terminal nucleotide overhanging segments of a 21-mer siRNA duplex having two-nucleotide 3'-overhangs with deoxyribonucleotides does not have an adverse effect on RNAi activity. Replacing up to four nucleotides on each end of the siRNA with deoxyribonucleotides has been reported to be well tolerated, whereas complete substitution with deoxyribonucleotides results in no RNAi activity (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877 and Tuschl *et al.*, International PCT Publication No. WO 01/75164). In addition, Elbashir *et al.*, *supra*, also report that substitution of siRNA with 2'-O-methyl nucleotides completely abolishes RNAi activity. Li *et al.*, International PCT Publication No. WO 00/44914, and Beach *et al.*, International PCT Publication No. WO 01/68836 preliminarily suggest that siRNA may include modifications to either the phosphate-sugar backbone or the nucleoside to include at least one of a nitrogen or sulfur heteroatom, however, neither application postulates to what extent such modifications would be tolerated in siRNA molecules, nor provides any further guidance or examples of such modified siRNA. Kreutzer *et al.*,

Canadian Patent Application No. 2,359,180, also describe certain chemical modifications for use in dsRNA constructs in order to counteract activation of double-stranded RNA-dependent protein kinase PKR, specifically 2'-amino or 2'-O-methyl nucleotides, and nucleotides containing a 2'-O or 4'-C methylene bridge. However, Kreutzer *et al.* similarly fails to provide examples or guidance as to what extent these modifications would be tolerated in dsRNA molecules.

Parrish *et al.*, 2000, *Molecular Cell*, 6, 1077-1087, tested certain chemical modifications targeting the unc-22 gene in *C. elegans* using long (>25 nt) siRNA transcripts. The authors describe the introduction of thiophosphate residues into these siRNA transcripts by incorporating thiophosphate nucleotide analogs with T7 and T3 RNA polymerase and observed that RNAs with two phosphorothioate modified bases also had substantial decreases in effectiveness as RNAi. Further, Parrish *et al.* reported that phosphorothioate modification of more than two residues greatly destabilized the RNAs *in vitro* such that interference activities could not be assayed. *Id.* at 1081. The authors also tested certain modifications at the 2'-position of the nucleotide sugar in the long siRNA transcripts and found that substituting deoxynucleotides for ribonucleotides produced a substantial decrease in interference activity, especially in the case of Uridine to Thymidine and/or Cytidine to deoxy-Cytidine substitutions. *Id.* In addition, the authors tested certain base modifications, including substituting, in sense and antisense strands of the siRNA, 4-thiouracil, 5-bromouracil, 5-iodouracil, and 3-(aminoallyl)uracil for uracil, and inosine for guanosine. Whereas 4-thiouracil and 5-bromouracil substitution appeared to be tolerated, Parrish reported that inosine produced a substantial decrease in interference activity when incorporated in either strand. Parrish also reported that incorporation of 5-iodouracil and 3-(aminoallyl)uracil in the antisense strand resulted in a substantial decrease in RNAi activity as well.

The use of longer dsRNA has been described. For example, Beach *et al.*, International PCT Publication No. WO 01/68836, describes specific methods for attenuating gene expression using endogenously-derived dsRNA. Tuschl *et al.*, International PCT Publication No. WO 01/75164, describe a *Drosophila in vitro* RNAi system and the use of specific siRNA molecules for certain functional genomic and certain therapeutic applications; although Tuschl, 2001, *Chem. Biochem.*, 2, 239-245, doubts that RNAi can be used to cure genetic diseases or viral infection due to the danger

of activating interferon response. Li *et al.*, International PCT Publication No. WO 00/44914, describe the use of specific long (141 bp-488 bp) enzymatically synthesized or vector expressed dsRNAs for attenuating the expression of certain target genes. Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646, describe certain methods for inhibiting the expression of particular genes in mammalian cells using certain long (550 bp-714 bp), enzymatically synthesized or vector expressed dsRNA molecules. Fire *et al.*, International PCT Publication No. WO 99/32619, describe particular methods for introducing certain long dsRNA molecules into cells for use in inhibiting gene expression in nematodes. Plaetinck *et al.*, International PCT Publication No. WO 00/01846, describe certain methods for identifying specific genes responsible for conferring a particular phenotype in a cell using specific long dsRNA molecules. Mello *et al.*, International PCT Publication No. WO 01/29058, describe the identification of specific genes involved in dsRNA-mediated RNAi. Pachuck *et al.*, International PCT Publication No. WO 00/63364, describe certain long (at least 200 nucleotide) dsRNA constructs. Deschamps Depaillette *et al.*, International PCT Publication No. WO 99/07409, describe specific compositions consisting of particular dsRNA molecules combined with certain anti-viral agents. Waterhouse *et al.*, International PCT Publication No. 99/53050 and 1998, *PNAS*, 95, 13959-13964, describe certain methods for decreasing the phenotypic expression of a nucleic acid in plant cells using certain dsRNAs. Driscoll *et al.*, International PCT Publication No. WO 01/49844, describe specific DNA expression constructs for use in facilitating gene silencing in targeted organisms.

Others have reported on various RNAi and gene-silencing systems. For example, Parrish *et al.*, 2000, *Molecular Cell*, 6, 1077-1087, describe specific chemically-modified dsRNA constructs targeting the unc-22 gene of *C. elegans*. Grossniklaus, International PCT Publication No. WO 01/38551, describes certain methods for regulating polycomb gene expression in plants using certain dsRNAs. Churikov *et al.*, International PCT Publication No. WO 01/42443, describe certain methods for modifying genetic characteristics of an organism using certain dsRNAs. Cogoni *et al.*, International PCT Publication No. WO 01/53475, describe certain methods for isolating a Neurospora silencing gene and uses thereof. Reed *et al.*, International PCT Publication No. WO 01/68836, describe certain methods for gene silencing in plants. Honer *et al.*,

International PCT Publication No. WO 01/70944, describe certain methods of drug screening using transgenic nematodes as Parkinson's Disease models using certain dsRNAs. Deak *et al.*, International PCT Publication No. WO 01/72774, describe certain *Drosophila*-derived gene products that may be related to RNAi in *Drosophila*. Arndt *et al.*, International PCT Publication No. WO 01/92513 describe certain methods for mediating gene suppression by using factors that enhance RNAi. Tuschl *et al.*, International PCT Publication No. WO 02/44321, describe certain synthetic siRNA constructs. Pachuk *et al.*, International PCT Publication No. WO 00/63364, and Satishchandran *et al.*, International PCT Publication No. WO 01/04313, describe certain methods and compositions for inhibiting the function of certain polynucleotide sequences using certain long (over 250 bp), vector expressed dsRNAs. Echeverri *et al.*, International PCT Publication No. WO 02/38805, describe certain *C. elegans* genes identified via RNAi. Kreutzer *et al.*, International PCT Publications Nos. WO 02/055692, WO 02/055693, and EP 1144623 B1 describes certain methods for inhibiting gene expression using dsRNA. Graham *et al.*, International PCT Publications Nos. WO 99/49029 and WO 01/70949, and AU 4037501 describe certain vector expressed siRNA molecules. Fire *et al.*, US 6,506,559, describe certain methods for inhibiting gene expression in vitro using certain long dsRNA (299 bp-1033 bp) constructs that mediate RNAi. Martinez *et al.*, 2002, *Cell*, 110, 563-574, describe certain single stranded siRNA constructs, including certain 5'-phosphorylated single stranded siRNAs that mediate RNA interference in Hela cells. Harborth *et al.*, 2003, Antisense & Nucleic Acid Drug Development, 13, 83-105, describe certain chemically and structurally modified siRNA molecules. Chiu and Rana, 2003, RNA, 9, 1034-1048, describe certain chemically and structurally modified siRNA molecules. Woolf *et al.*, International PCT Publication Nos. WO 03/064626 and WO 03/064625 describe certain chemically modified dsRNA constructs.

SUMMARY OF THE INVENTION

This invention relates to compounds, compositions, and methods useful for modulating the expression of genes associated with inhibitor of apoptosis proteins (IAPs), for example, XIAP (X-linked inhibitor of apoptosis protein) and related genes, such as HIAP1 (human inhibitor or apoptosis 1) , HIAP2 (human inhibitor or apoptosis 2), NAIP (neuronal apoptosis inhibitor protein) and other IAP's (inhibitors of apoptosis

proteins) using short interfering nucleic acid (siNA) molecules. This invention also relates to compounds, compositions, and methods useful for modulating the expression and activity of other genes involved in pathways of XIAP gene expression and/or activity by RNA interference (RNAi) using small nucleic acid molecules. In particular, the instant invention features small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules and methods used to modulate the expression of XIAP genes.

A siNA of the invention can be unmodified or chemically-modified. A siNA of the instant invention can be chemically synthesized, expressed from a vector or enzymatically synthesized. The instant invention also features various chemically-modified synthetic short interfering nucleic acid (siNA) molecules capable of modulating XIAP gene expression or activity in cells by RNA interference (RNAi). The use of chemically-modified siNA improves various properties of native siNA molecules through increased resistance to nuclease degradation *in vivo* and/or through improved cellular uptake. Further, contrary to earlier published studies, siNA having multiple chemical modifications retains its RNAi activity. The siNA molecules of the instant invention provide useful reagents and methods for a variety of therapeutic, diagnostic, target validation, genomic discovery, genetic engineering, and pharmacogenomic applications.

In one embodiment, the invention features one or more siNA molecules and methods that independently or in combination modulate the expression of XIAP, HIAP1, HIAP2, and/or NAIP gene(s) encoding proteins, such as proteins comprising XIAP, HIAP1, HIAP2, and/or NAIP associated with the maintenance and/or development of cancer and other proliferative disorders, such as ovarian cancer; cancers of non-lymphoid parenchymal organs including the heart, placenta, skeletal muscle and lung; breast cancer; cancers of the head and neck, including various lymphomas such as mantle cell lymphoma; non-Hodgkins lymphoma; adenoma; squamous cell carcinoma; laryngeal carcinoma; cancers of the retina; cancers of the esophagus; multiple myeloma; melanoma; colorectal cancer; lung cancer; bladder cancer; prostate cancer; glioblastoma; and proliferative diseases and conditions such as restenosis and polycystic kidney disease; and any other indications that can respond to the level of a XIAP gene in a cell

or tissue, for example, genes encoding sequences comprising those sequences referred to by GenBank Accession Nos. shown in **Table I**, referred to herein generally as XIAP. The description below of the various aspects and embodiments of the invention is provided with reference to exemplary XIAP gene referred to herein as XIAP, which is also known as BIRC4. However, the various aspects and embodiments are also directed to other apoptosis inhibotor genes such as HIAP1, HIAP2, and NAIP, and other XIAP genes, such XIAP homolog genes, XIAP transcript variants and polymorphisms (e.g., single nucleotide polymorphism, (SNPs)) associated with certain XIAP genes, including genes encoding any XIAP ligands and receptors. As such, the various aspects and embodiments are also directed to other genes, such as HIAP1, HIAP2, AND NAIP, that are involved in XIAP mediated pathways of signal transduction or gene expression that are involved in, for example, the progression, development, and/or maintenance of disease, such as cancer and other proliferative disorders (e.g., ovarian cancer; cancers of non-lymphoid parenchymal organs including the heart, placenta, skeletal muscle and lung; breast cancer; cancers of the head and neck, including various lymphomas such as mantle cell lymphoma; non-Hodgkins lymphoma; adenoma; squamous cell carcinoma; laryngeal carcinoma; cancers of the retina; cancers of the esophagus; multiple myeloma; melanoma; colorectal cancer; lung cancer; bladder cancer; prostate cancer; glioblastoma; and proliferative diseases and conditions such as restenosis and polycystic kidney disease; and any other indications that can respond to the level of a XIAP gene in a cell or tissue). These additional genes can be analyzed for target sites using the methods described for XIAP genes herein. Thus, the modulation of other genes and the effects of such modulation of the other genes can be performed, determined, and measured as described herein.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a XIAP gene, wherein said siNA molecule comprises about 15 to about 28 base pairs.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a XIAP RNA via RNA interference (RNAi), wherein the double stranded siNA molecule comprises a first and a second strand, each strand of the siNA molecule is about 18 to about 28 nucleotides in length, the first strand of the siNA molecule comprises nucleotide sequence having sufficient

complementarity to the XIAP RNA for the siNA molecule to direct cleavage of the XIAP RNA via RNA interference, and the second strand of said siNA molecule comprises nucleotide sequence that is complementary to the first strand.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a XIAP RNA via RNA interference (RNAi), wherein the double stranded siNA molecule comprises a first and a second strand, each strand of the siNA molecule is about 18 to about 23 nucleotides in length, the first strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the XIAP RNA for the siNA molecule to direct cleavage of the XIAP RNA via RNA interference, and the second strand of said siNA molecule comprises nucleotide sequence that is complementary to the first strand.

In one embodiment, the invention features a chemically synthesized double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a XIAP RNA via RNA interference (RNAi), wherein each strand of the siNA molecule is about 18 to about 28 nucleotides in length; and one strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the XIAP RNA for the siNA molecule to direct cleavage of the XIAP RNA via RNA interference.

In one embodiment, the invention features a chemically synthesized double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a XIAP RNA via RNA interference (RNAi), wherein each strand of the siNA molecule is about 18 to about 23 nucleotides in length; and one strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the XIAP RNA for the siNA molecule to direct cleavage of the XIAP RNA via RNA interference.

In one embodiment, the invention features a siNA molecule that down-regulates expression of a XIAP gene, for example, wherein the XIAP gene comprises XIAP encoding sequence. In one embodiment, the invention features a siNA molecule that down-regulates expression of a XIAP gene, for example, wherein the XIAP gene comprises XIAP non-coding sequence or regulatory elements involved in XIAP gene expression.

In one embodiment, a siNA of the invention is used to inhibit the expression of XIAP genes or a XIAP gene family, wherein the genes or gene family sequences share sequence homology. Such homologous sequences can be identified as is known in the art, for example using sequence alignments. siNA molecules can be designed to target such homologous sequences, for example using perfectly complementary sequences or by incorporating non-canonical base pairs, for example mismatches and/or wobble base pairs, that can provide additional target sequences. In instances where mismatches are identified, non-canonical base pairs (for example, mismatches and/or wobble bases) can be used to generate siNA molecules that target more than one gene sequence. In a non-limiting example, non-canonical base pairs such as UU and CC base pairs are used to generate siNA molecules that are capable of targeting sequences for differing XIAP targets that share sequence homology. As such, one advantage of using siNAs of the invention is that a single siNA can be designed to include nucleic acid sequence that is complementary to the nucleotide sequence that is conserved between the homologous genes. In this approach, a single siNA can be used to inhibit expression of more than one gene instead of using more than one siNA molecule to target the different genes.

In one embodiment, the invention features a siNA molecule having RNAi activity against XIAP RNA, wherein the siNA molecule comprises a sequence complementary to any RNA having XIAP encoding sequence, such as those sequences having GenBank Accession Nos. shown in **Table I.** In another embodiment, the invention features a siNA molecule having RNAi activity against XIAP RNA, wherein the siNA molecule comprises a sequence complementary to an RNA having variant XIAP encoding sequence, for example other mutant XIAP genes not shown in **Table I** but known in the art to be associated with the maintenance and/or development of development of cancer and other proliferative disorders, such as ovarian cancer; cancers of non-lymphoid parenchymal organs including the heart, placenta, skeletal muscle and lung; breast cancer; cancers of the head and neck, including various lymphomas such as mantle cell lymphoma; non-Hodgkins lymphoma; adenoma; squamous cell carcinoma; laryngeal carcinoma; cancers of the retina; cancers of the esophagus; multiple myeloma; melanoma; colorectal cancer; lung cancer; bladder cancer; prostate cancer; glioblastoma; and proliferative diseases and conditions such as restenosis and polycystic kidney disease; and any other indications that can respond to the level of a XIAP gene in a cell

or tissue. Chemical modifications as shown in **Tables III and IV** or otherwise described herein can be applied to any siNA construct of the invention. In another embodiment, a siNA molecule of the invention includes a nucleotide sequence that can interact with nucleotide sequence of a XIAP gene and thereby mediate silencing of XIAP gene expression, for example, wherein the siNA mediates regulation of XIAP gene expression by cellular processes that modulate the chromatin structure or methylation patterns of the XIAP gene and prevent transcription of the XIAP gene.

In one embodiment, siNA molecules of the invention are used to down regulate or inhibit the expression of XIAP proteins arising from XIAP haplotype polymorphisms that are associated with a disease or condition, (e.g., cancer, such as ovarian cancer; cancers of non-lymphoid parenchymal organs including the heart, placenta, skeletal muscle and lung; breast cancer; cancers of the head and neck, including various lymphomas such as mantle cell lymphoma; non-Hodgkins lymphoma; adenoma; squamous cell carcinoma; laryngeal carcinoma; cancers of the retina; cancers of the esophagus; multiple myeloma; melanoma; colorectal cancer; lung cancer; bladder cancer; prostate cancer; glioblastoma; and proliferative diseases and conditions such as restenosis and polycystic kidney disease). Analysis of XIAP genes, or XIAP protein or RNA levels can be used to identify subjects with such polymorphisms or those subjects who are at risk of developing traits, conditions, or diseases described herein. These subjects are amenable to treatment, for example, treatment with siNA molecules of the invention and any other composition useful in treating diseases related to XIAP gene expression. As such, analysis of XIAP protein or RNA levels can be used to determine treatment type and the course of therapy in treating a subject. Monitoring of XIAP protein or RNA levels can be used to predict treatment outcome and to determine the efficacy of compounds and compositions that modulate the level and/or activity of certain XIAP proteins associated with a trait, condition, or disease.

In one embodiment of the invention a siNA molecule comprises an antisense strand comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof encoding a XIAP protein. The siNA further comprises a sense strand, wherein said sense strand comprises a nucleotide sequence of a XIAP gene or a portion thereof.

In another embodiment, a siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence encoding a XIAP protein or a portion thereof. The siNA molecule further comprises a sense region, wherein said sense region comprises a nucleotide sequence of a XIAP gene or a portion thereof.

In another embodiment, the invention features a siNA molecule comprising a nucleotide sequence in the antisense region of the siNA molecule that is complementary to a nucleotide sequence or portion of sequence of a XIAP gene. In another embodiment, the invention features a siNA molecule comprising a region, for example, the antisense region of the siNA construct, complementary to a sequence comprising a XIAP gene sequence or a portion thereof.

In one embodiment, the antisense region of XIAP siNA constructs comprises a sequence complementary to sequence having any of SEQ ID NOs. 1-467 or 935-942. In one embodiment, the antisense region of XIAP constructs comprises sequence having any of SEQ ID NOs. 468-934, 951-958, 967-974, 983-990, 999-1006, 1015-1038,1040, 1042, 1044, 1047, 1049, 1051, 1053, or 1056. In another embodiment, the sense region of XIAP constructs comprises sequence having any of SEQ ID NOs. 1-467, 935-950, 959-966, 975-982, 991-998, 1007-1014, 1039, 1041, 1043, 1045, 1046, 1048, 1050, 1052, 1054, or 1055.

In one embodiment, a siNA molecule of the invention comprises any of SEQ ID NOs. 1-1056. The sequences shown in SEQ ID NOs: 1-1056 are not limiting. A siNA molecule of the invention can comprise any contiguous XIAP sequence (e.g., about 15 to about 25 or more, or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 or more contiguous XIAP nucleotides).

In yet another embodiment, the invention features a siNA molecule comprising a sequence, for example, the antisense sequence of the siNA construct, complementary to a sequence or portion of sequence comprising sequence represented by GenBank Accession Nos. shown in **Table I**. Chemical modifications in **Tables III and IV** and described herein can be applied to any siNA construct of the invention.

In one embodiment of the invention a siNA molecule comprises an antisense strand having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein the antisense strand is complementary to a RNA sequence or a portion thereof encoding a XIAP protein, and wherein said siNA further comprises a sense strand having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, and wherein said sense strand and said antisense strand are distinct nucleotide sequences where at least about 15 nucleotides in each strand are complementary to the other strand.

In another embodiment of the invention a siNA molecule of the invention comprises an antisense region having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein the antisense region is complementary to a RNA sequence encoding a XIAP protein, and wherein said siNA further comprises a sense region having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein said sense region and said antisense region are comprised in a linear molecule where the sense region comprises at least about 15 nucleotides that are complementary to the antisense region.

In one embodiment, a siNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by a XIAP gene. Because XIAP genes can share some degree of sequence homology with each other, siNA molecules can be designed to target a class of XIAP genes or alternately specific XIAP genes (e.g., polymorphic variants) by selecting sequences that are either shared amongst different XIAP targets or alternatively that are unique for a specific XIAP target. Therefore, in one embodiment, the siNA molecule can be designed to target conserved regions of XIAP RNA sequences having homology among several XIAP gene variants so as to target a class of XIAP genes with one siNA molecule. Accordingly, in one embodiment, the siNA molecule of the invention modulates the expression of one or both XIAP alleles in a subject. In another embodiment, the siNA molecule can be designed to target a sequence that is unique to a specific XIAP RNA sequence (e.g., a single XIAP allele or XIAP single nucleotide polymorphism (SNP)) due to the high degree of specificity that the siNA molecule requires to mediate RNAi activity.

In one embodiment, nucleic acid molecules of the invention that act as mediators of the RNA interference gene silencing response are double-stranded nucleic acid molecules. In another embodiment, the siNA molecules of the invention consist of duplex nucleic acid molecules containing about 15 to about 30 base pairs between oligonucleotides comprising about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides. In yet another embodiment, siNA molecules of the invention comprise duplex nucleic acid molecules with overhanging ends of about 1 to about 3 (*e.g.*, about 1, 2, or 3) nucleotides, for example, about 21-nucleotide duplexes with about 19 base pairs and 3'-terminal mononucleotide, dinucleotide, or trinucleotide overhangs. In yet another embodiment, siNA molecules of the invention comprise duplex nucleic acid molecules with blunt ends, where both ends are blunt, or alternatively, where one of the ends is blunt.

In one embodiment, the invention features one or more chemically-modified siNA constructs having specificity for XIAP expressing nucleic acid molecules, such as RNA encoding a XIAP protein. In one embodiment, the invention features a RNA based siNA molecule (e.g., a siNA comprising 2'-OH nucleotides) having specificity for XIAP expressing nucleic acid molecules that includes one or more chemical modifications described herein. Non-limiting examples of such chemical modifications include without limitation phosphorothioate internucleotide linkages, 2'-deoxyribonucleotides, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, "universal base" nucleotides, "acyclic" nucleotides, 5-C-methyl nucleotides, and terminal glyceryl and/or inverted deoxy abasic residue incorporation. These chemical modifications, when used in various siNA constructs, (e.g., RNA based siNA constructs), are shown to preserve RNAi activity in cells while at the same time, dramatically increasing the serum stability of these compounds. Furthermore, contrary to the data published by Parrish *et al., supra,* applicant demonstrates that multiple (greater than one) phosphorothioate substitutions are well-tolerated and confer substantial increases in serum stability for modified siNA constructs.

In one embodiment, a siNA molecule of the invention comprises modified nucleotides while maintaining the ability to mediate RNAi. The modified nucleotides

can be used to improve *in vitro* or *in vivo* characteristics such as stability, activity, and/or bioavailability. For example, a siNA molecule of the invention can comprise modified nucleotides as a percentage of the total number of nucleotides present in the siNA molecule. As such, a siNA molecule of the invention can generally comprise about 5% to about 100% modified nucleotides (e.g., about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides). The actual percentage of modified nucleotides present in a given siNA molecule will depend on the total number of nucleotides present in the siNA. If the siNA molecule is single stranded, the percent modification can be based upon the total number of nucleotides present in the single stranded siNA molecules. Likewise, if the siNA molecule is double stranded, the percent modification can be based upon the total number of nucleotides present in the sense strand, antisense strand, or both the sense and antisense strands.

One aspect of the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a XIAP gene. In one embodiment, the double stranded siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is about 21 nucleotides long. In one embodiment, the double-stranded siNA molecule does not contain any ribonucleotides. In another embodiment, the double-stranded siNA molecule comprises one or more ribonucleotides. In one embodiment, each strand of the double-stranded siNA molecule independently comprises about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein each strand comprises about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to the nucleotides of the other strand. In one embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the XIAP gene, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence of the XIAP gene or a portion thereof.

In another embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a XIAP gene comprising an antisense region, wherein the antisense region comprises a nucleotide sequence that is

complementary to a nucleotide sequence of the XIAP gene or a portion thereof, and a sense region, wherein the sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence of the XIAP gene or a portion thereof. In one embodiment, the antisense region and the sense region independently comprise about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein the antisense region comprises about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to nucleotides of the sense region.

In another embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a XIAP gene comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the XIAP gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region.

In one embodiment, a siNA molecule of the invention comprises blunt ends, i.e., ends that do not include any overhanging nucleotides. For example, a siNA molecule comprising modifications described herein (e.g., comprising nucleotides having Formulae I-VII or siNA constructs comprising "Stab 00"-"Stab 28" **(Table IV)** or any combination thereof (see **Table IV**)) and/or any length described herein can comprise blunt ends or ends with no overhanging nucleotides.

In one embodiment, any siNA molecule of the invention can comprise one or more blunt ends, i.e. where a blunt end does not have any overhanging nucleotides. In one embodiment, the blunt ended siNA molecule has a number of base pairs equal to the number of nucleotides present in each strand of the siNA molecule. In another embodiment, the siNA molecule comprises one blunt end, for example wherein the 5'-end of the antisense strand and the 3'-end of the sense strand do not have any overhanging nucleotides. In another example, the siNA molecule comprises one blunt end, for example wherein the 3'-end of the antisense strand and the 5'-end of the sense strand do not have any overhanging nucleotides. In another example, a siNA molecule comprises two blunt ends, for example wherein the 3'-end of the antisense strand and the 5'-end of the sense strand as well as the 5'-end of the antisense strand and 3'-end of the

sense strand do not have any overhanging nucleotides. A blunt ended siNA molecule can comprise, for example, from about 15 to about 30 nucleotides (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides). Other nucleotides present in a blunt ended siNA molecule can comprise, for example, mismatches, bulges, loops, or wobble base pairs to modulate the activity of the siNA molecule to mediate RNA interference.

By "blunt ends" is meant symmetric termini or termini of a double stranded siNA molecule having no overhanging nucleotides. The two strands of a double stranded siNA molecule align with each other without over-hanging nucleotides at the termini. For example, a blunt ended siNA construct comprises terminal nucleotides that are complementary between the sense and antisense regions of the siNA molecule.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a XIAP gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. The sense region can be connected to the antisense region via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker.

In one embodiment, the invention features double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a XIAP gene, wherein the siNA molecule comprises about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein each strand of the siNA molecule comprises one or more chemical modifications. In another embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of a XIAP gene or a portion thereof, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of the XIAP gene. In another embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of a XIAP gene or portion thereof, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or portion thereof of

the XIAP gene. In another embodiment, each strand of the siNA molecule comprises about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, and each strand comprises at least about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to the nucleotides of the other strand. The XIAP gene can comprise, for example, sequences referred to in **Table I**.

In one embodiment, a siNA molecule of the invention comprises no ribonucleotides. In another embodiment, a siNA molecule of the invention comprises ribonucleotides.

In one embodiment, a siNA molecule of the invention comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence of a XIAP gene or a portion thereof, and the siNA further comprises a sense region comprising a nucleotide sequence substantially similar to the nucleotide sequence of the XIAP gene or a portion thereof. In another embodiment, the antisense region and the sense region each comprise about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides and the antisense region comprises at least about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to nucleotides of the sense region. The XIAP gene can comprise, for example, sequences referred to in **Table I**. In another embodiment, the siNA is a double stranded nucleic acid molecule, where each of the two strands of the siNA molecule independently comprise about 15 to about 40 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 23, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides, and where one of the strands of the siNA molecule comprises at least about 15 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 or more) nucleotides that are complementary to the nucleic acid sequence of the XIAP gene or a portion thereof.

In one embodiment, a siNA molecule of the invention comprises a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by a XIAP gene, or a portion thereof, and the sense region comprises a nucleotide sequence that is complementary to the antisense region. In one embodiment, the siNA molecule is

assembled from two separate oligonucleotide fragments, wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. In another embodiment, the sense region is connected to the antisense region via a linker molecule. In another embodiment, the sense region is connected to the antisense region via a linker molecule, such as a nucleotide or non-nucleotide linker. The XIAP gene can comprise, for example, sequences referred in to **Table I.**

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a XIAP gene comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the XIAP gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein the siNA molecule has one or more modified pyrimidine and/or purine nucleotides. In one embodiment, the pyrimidine nucleotides in the sense region are 2'-O-methyl pyrimidine nucleotides or 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In another embodiment, the pyrimidine nucleotides in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides. In another embodiment, the pyrimidine nucleotides in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In one embodiment, the pyrimidine nucleotides in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the antisense region are 2'-O-methyl or 2'-deoxy purine nucleotides. In another embodiment of any of the above-described siNA molecules, any nucleotides present in a non-complementary region of the sense strand (e.g. overhang region) are 2'-deoxy nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a XIAP gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule, and wherein the fragment comprising the sense

region includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the fragment. In one embodiment, the terminal cap moiety is an inverted deoxy abasic moiety or glyceryl moiety. In one embodiment, each of the two fragments of the siNA molecule independently comprise about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides. In another embodiment, each of the two fragments of the siNA molecule independently comprise about 15 to about 40 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 23, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides. In a non-limiting example, each of the two fragments of the siNA molecule comprise about 21 nucleotides.

In one embodiment, the invention features a siNA molecule comprising at least one modified nucleotide, wherein the modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide. The siNA can be, for example, about 15 to about 40 nucleotides in length. In one embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In one embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine or 2'-deoxy-2'-fluoro uridine nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro cytidine nucleotides. In one embodiment, all adenosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro adenosine nucleotides. In one embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro guanosine nucleotides. The siNA can further comprise at least one modified internucleotidic linkage, such as phosphorothioate linkage. In one embodiment, the 2'-deoxy-2'-fluoronucleotides are present at specifically selected locations in the siNA that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine nucleotides.

In one embodiment, the invention features a method of increasing the stability of a siNA molecule against cleavage by ribonucleases comprising introducing at least one modified nucleotide into the siNA molecule, wherein the modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide. In one embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In one embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine or 2'-

deoxy-2'-fluoro uridine nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro cytidine nucleotides. In one embodiment, all adenosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro adenosine nucleotides. In one embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro guanosine nucleotides. The siNA can further comprise at least one modified internucleotidic linkage, such as phosphorothioate linkage. In one embodiment, the 2'-deoxy-2'-fluoronucleotides are present at specifically selected locations in the siNA that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a XIAP gene comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the XIAP gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein the purine nucleotides present in the antisense region comprise 2'-deoxy- purine nucleotides. In an alternative embodiment, the purine nucleotides present in the antisense region comprise 2'-O-methyl purine nucleotides. In either of the above embodiments, the antisense region can comprise a phosphorothioate internucleotide linkage at the 3' end of the antisense region. Alternatively, in either of the above embodiments, the antisense region can comprise a glyceryl modification at the 3' end of the antisense region. In another embodiment of any of the above-described siNA molecules, any nucleotides present in a non-complementary region of the antisense strand (e.g. overhang region) are 2'-deoxy nucleotides.

In one embodiment, the antisense region of a siNA molecule of the invention comprises sequence complementary to a portion of a XIAP transcript having sequence unique to a particular XIAP disease related allele, such as sequence comprising a single nucleotide polymorphism (SNP) associated with the disease specific allele. As such, the antisense region of a siNA molecule of the invention can comprise sequence

complementary to sequences that are unique to a particular allele to provide specificity in mediating selective RNAi against the disease, condition, or trait related allele.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a XIAP gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule, where each strand is about 21 nucleotides long and where about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule, wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule, where each strand is about 19 nucleotide long and where the nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule to form at least about 15 (e.g., 15, 16, 17, 18, or 19) base pairs, wherein one or both ends of the siNA molecule are blunt ends. In one embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine nucleotide, such as a 2'-deoxy-thymidine. In another embodiment, all nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule of about 19 to about 25 base pairs having a sense region and an antisense region, where about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the XIAP gene. In another embodiment, about 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the XIAP gene. In any of the above embodiments, the 5'-end of the fragment comprising said antisense region can optionally include a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a XIAP RNA sequence (e.g., wherein said target RNA sequence is encoded by a XIAP gene involved in the XIAP pathway), wherein the siNA molecule does not contain any ribonucleotides and wherein

each strand of the double-stranded siNA molecule is about 15 to about 30 nucleotides. In one embodiment, the siNA molecule is 21 nucleotides in length. Examples of non-ribonucleotide containing siNA constructs are combinations of stabilization chemistries shown in **Table IV** in any combination of Sense/Antisense chemistries, such as Stab 7/8, Stab 7/11, Stab 8/8, Stab 18/8, Stab 18/11, Stab 12/13, Stab 7/13, Stab 18/13, Stab 7/19, Stab 8/19, Stab 18/19, Stab 7/20, Stab 8/20, or Stab 18/20 (e.g., any siNA having Stab 7, 8, 11, 12, 13, 14, 15, 17, 18, 19, or 20 sense or antisense strands or any combination thereof).

In one embodiment, the invention features a chemically synthesized double stranded RNA molecule that directs cleavage of a XIAP RNA via RNA interference, wherein each strand of said RNA molecule is about 15 to about 30 nucleotides in length; one strand of the RNA molecule comprises nucleotide sequence having sufficient complementarity to the XIAP RNA for the RNA molecule to direct cleavage of the XIAP RNA via RNA interference; and wherein at least one strand of the RNA molecule optionally comprises one or more chemically modified nucleotides described herein, such as without limitation deoxynucleotides, 2'-O-methyl nucleotides, 2'-deoxy-2'-fluoro nucloetides, 2'-O-methoxyethyl nucleotides etc.

In one embodiment, the invention features a medicament comprising a siNA molecule of the invention.

In one embodiment, the invention features an active ingredient comprising a siNA molecule of the invention.

In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule to inhibit, down-regulate, or reduce expression of a XIAP gene, wherein the siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is independently about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 or more) nucleotides long. In one embodiment, the siNA molecule of the invention is a double stranded nucleic acid molecule comprising one or more chemical modifications, where each of the two fragments of the siNA molecule independently comprise about 15 to about 40 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 23, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides and where one of the strands comprises

at least 15 nucleotides that are complementary to nucleotide sequence of XIAP encoding RNA or a portion thereof. In a non-limiting example, each of the two fragments of the siNA molecule comprise about 21 nucleotides. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule comprising one or more chemical modifications, where each strand is about 21 nucleotide long and where about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule, wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule comprising one or more chemical modifications, where each strand is about 19 nucleotide long and where the nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule to form at least about 15 (e.g., 15, 16, 17, 18, or 19) base pairs, wherein one or both ends of the siNA molecule are blunt ends. In one embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine nucleotide, such as a 2'-deoxy-thymidine. In another embodiment, all nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule of about 19 to about 25 base pairs having a sense region and an antisense region and comprising one or more chemical modifications, where about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the XIAP gene. In another embodiment, about 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the XIAP gene. In any of the above embodiments, the 5'-end of the fragment comprising said antisense region can optionally include a phosphate group.

In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits, down-regulates, or reduces expression of a XIAP gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of XIAP RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a

nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits, down-regulates, or reduces expression of a XIAP gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of XIAP RNA or a portion thereof, wherein the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits , down-regulates, or reduces expression of a XIAP gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of XIAP RNA that encodes a protein or portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In one embodiment, each strand of the siNA molecule comprises about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides, wherein each strand comprises at least about 15 nucleotides that are complementary to the nucleotides of the other strand. In one embodiment, the siNA molecule is assembled from two oligonucleotide fragments, wherein one fragment comprises the nucleotide sequence of the antisense strand of the siNA molecule and a second fragment comprises nucleotide sequence of the sense region of the siNA molecule. In one embodiment, the sense strand is connected to the antisense strand via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker. In a further embodiment, the pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In another embodiment, the pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'fluoro pyrimidine nucleotides and the purine

nucleotides present in the sense region are 2'-O-methyl purine nucleotides. In still another embodiment, the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-deoxy purine nucleotides. In another embodiment, the antisense strand comprises one or more 2'-deoxy-2'-fluoro pyrimidine nucleotides and one or more 2'-O-methyl purine nucleotides. In another embodiment, the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-O-methyl purine nucleotides. In a further embodiment the sense strand comprises a 3'-end and a 5'-end, wherein a terminal cap moiety (e.g., an inverted deoxy abasic moiety or inverted deoxy nucleotide moiety such as inverted thymidine) is present at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the sense strand. In another embodiment, the antisense strand comprises a phosphorothioate internucleotide linkage at the 3' end of the antisense strand. In another embodiment, the antisense strand comprises a glyceryl modification at the 3' end. In another embodiment, the 5'-end of the antisense strand optionally includes a phosphate group.

In any of the above-described embodiments of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a XIAP gene, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, each of the two strands of the siNA molecule can comprise about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides. In one embodiment, about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule. In another embodiment, about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule, wherein at least two 3' terminal nucleotides of each strand of the siNA molecule are not base-paired to the nucleotides of the other strand of the siNA molecule. In another embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine, such as 2'-deoxy-thymidine. In one embodiment,

each strand of the siNA molecule is base-paired to the complementary nucleotides of the other strand of the siNA molecule. In one embodiment, about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides of the antisense strand are base-paired to the nucleotide sequence of the XIAP RNA or a portion thereof. In one embodiment, about 18 to about 25 (e.g., about 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides of the antisense strand are base-paired to the nucleotide sequence of the XIAP RNA or a portion thereof.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a XIAP gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of XIAP RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the 5'-end of the antisense strand optionally includes a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a XIAP gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of XIAP RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence or a portion thereof of the antisense strand is complementary to a nucleotide sequence of the untranslated region or a portion thereof of the XIAP RNA.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a XIAP gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of XIAP RNA or a portion thereof, wherein the other strand is a sense strand which comprises nucleotide

sequence that is complementary to a nucleotide sequence of the antisense strand, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence of the antisense strand is complementary to a nucleotide sequence of the XIAP or a portion thereof that is present in the XIAP RNA.

In one embodiment, the invention features a composition comprising a siNA molecule of the invention in a pharmaceutically acceptable carrier or diluent.

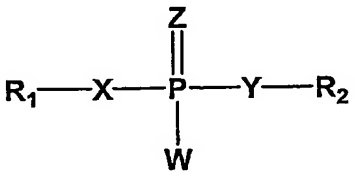
In a non-limiting example, the introduction of chemically-modified nucleotides into nucleic acid molecules provides a powerful tool in overcoming potential limitations of *in vivo* stability and bioavailability inherent to native RNA molecules that are delivered exogenously. For example, the use of chemically-modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given therapeutic effect since chemically-modified nucleic acid molecules tend to have a longer half-life in serum. Furthermore, certain chemical modifications can improve the bioavailability of nucleic acid molecules by targeting particular cells or tissues and/or improving cellular uptake of the nucleic acid molecule. Therefore, even if the activity of a chemically-modified nucleic acid molecule is reduced as compared to a native nucleic acid molecule, for example, when compared to an all-RNA nucleic acid molecule, the overall activity of the modified nucleic acid molecule can be greater than that of the native molecule due to improved stability and/or delivery of the molecule. Unlike native unmodified siNA, chemically-modified siNA can also minimize the possibility of activating interferon activity in humans.

In any of the embodiments of siNA molecules described herein, the antisense region of a siNA molecule of the invention can comprise a phosphorothioate internucleotide linkage at the 3'-end of said antisense region. In any of the embodiments of siNA molecules described herein, the antisense region can comprise about one to about five phosphorothioate internucleotide linkages at the 5'-end of said antisense region. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs of a siNA molecule of the invention can comprise ribonucleotides or deoxyribonucleotides that are chemically-modified at a nucleic acid sugar, base, or backbone. In any of the embodiments of siNA molecules described herein, the 3'-

terminal nucleotide overhangs can comprise one or more universal base ribonucleotides. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs can comprise one or more acyclic nucleotides.

One embodiment of the invention provides an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention in a manner that allows expression of the nucleic acid molecule. Another embodiment of the invention provides a mammalian cell comprising such an expression vector. The mammalian cell can be a human cell. The siNA molecule of the expression vector can comprise a sense region and an antisense region. The antisense region can comprise sequence complementary to a RNA or DNA sequence encoding XIAP and the sense region can comprise sequence complementary to the antisense region. The siNA molecule can comprise two distinct strands having complementary sense and antisense regions. The siNA molecule can comprise a single strand having complementary sense and antisense regions.

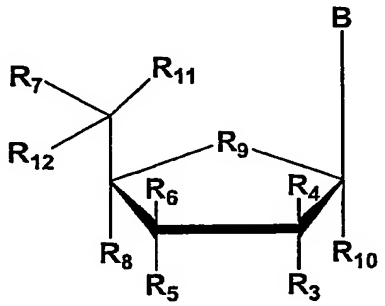
In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against XIAP inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides comprising a backbone modified internucleotide linkage having Formula I:



wherein each R1 and R2 is independently any nucleotide, non-nucleotide, or polynucleotide which can be naturally-occurring or chemically-modified, each X and Y is independently O, S, N, alkyl, or substituted alkyl, each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, or acetyl and wherein W, X, Y, and Z are optionally not all O. In another embodiment, a backbone modification of the invention comprises a phosphonoacetate and/or thiophosphonoacetate internucleotide linkage (see for example Sheehan et al., 2003, Nucleic Acids Research, 31, 4109-4118).

The chemically-modified internucleotide linkages having Formula I, for example, wherein any Z, W, X, and/or Y independently comprises a sulphur atom, can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) chemically-modified internucleotide linkages having Formula I at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified internucleotide linkages having Formula I at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In another embodiment, a siNA molecule of the invention having internucleotide linkage(s) of Formula I also comprises a chemically-modified nucleotide or non-nucleotide having any of Formulae I-VII.

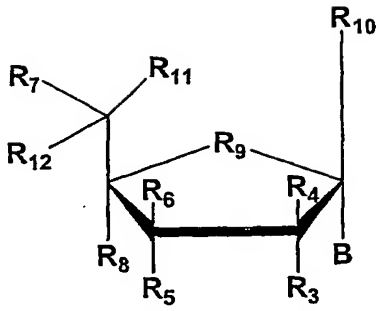
In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against XIAP inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula II:



wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula II can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotide or non-nucleotide of Formula II at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 5'-end of the sense strand, the antisense strand, or both strands. In anther non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 3'-end of the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against XIAP inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula III:

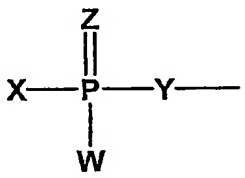


wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be employed to be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula III can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide(s) or non-nucleotide(s) of Formula III at the 5'-end of the sense strand, the antisense strand, or both strands. In anther non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end of the sense strand, the antisense strand, or both strands.

In another embodiment, a siNA molecule of the invention comprises a nucleotide having Formula II or III, wherein the nucleotide having Formula II or III is in an inverted configuration. For example, the nucleotide having Formula II or III is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against XIAP inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a 5'-terminal phosphate group having Formula IV:



wherein each X and Y is independently O, S, N, alkyl, substituted alkyl, or alkylhalo; wherein each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, alkylhalo, or acetyl; and wherein W, X, Y and Z are not all O.

In one embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand, for example, a strand complementary to a target RNA, wherein the siNA molecule comprises an all RNA siNA molecule. In another embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand wherein the siNA molecule also comprises about 1 to about 3 (*e.g.*, about 1, 2, or 3) nucleotide 3'-terminal nucleotide overhangs having about 1 to about 4 (*e.g.*, about 1, 2, 3, or 4) deoxyribonucleotides on the 3'-end of one or both strands. In another embodiment, a 5'-terminal phosphate group having Formula IV is present on the target-complementary strand of a siNA molecule of the invention, for example a siNA molecule having chemical modifications having any of Formulae I-VII.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against XIAP inside a cell or reconstituted *in vitro* system, wherein the chemical modification

comprises one or more phosphorothioate internucleotide linkages. For example, in a non-limiting example, the invention features a chemically-modified short interfering nucleic acid (siNA) having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in one siNA strand. In yet another embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) individually having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in both siNA strands. The phosphorothioate internucleotide linkages can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more phosphorothioate internucleotide linkages at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) consecutive phosphorothioate internucleotide linkages at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a siNA molecule, wherein the sense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or about one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the

antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the sense strand comprises about 1 to about 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5 or more, for example about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a siNA molecule, wherein the antisense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or about one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate

internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3' and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5, for example about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule having about 1 to about 5 or more (specifically about 1, 2, 3, 4, 5 or more) phosphorothioate internucleotide linkages in each strand of the siNA molecule.

In another embodiment, the invention features a siNA molecule comprising 2'-5' internucleotide linkages. The 2'-5' internucleotide linkage(s) can be at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of one or both siNA sequence strands. In addition, the 2'-5' internucleotide linkage(s) can be present at various other positions within one or both siNA sequence strands, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a pyrimidine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a purine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage.

In another embodiment, a chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified, wherein each strand is independently about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length, wherein the duplex has about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein the chemical modification comprises a structure having any of Formulae I-VII. For example, an exemplary chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein each strand consists of about 21 nucleotides, each having a 2-nucleotide 3'-terminal nucleotide overhang, and wherein the duplex has about 19 base pairs. In another embodiment, a siNA molecule of the invention comprises a single stranded hairpin structure, wherein the siNA is about 36 to about 70 (*e.g.*, about 36, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein the siNA can include a chemical modification comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 42 to about 50 (*e.g.*, about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 19 to about 21 (e.g., 19, 20, or 21) base pairs and a 2-nucleotide 3'-terminal nucleotide overhang. In another embodiment, a linear hairpin

siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. For example, a linear hairpin siNA molecule of the invention is designed such that degradation of the loop portion of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In another embodiment, a siNA molecule of the invention comprises a hairpin structure, wherein the siNA is about 25 to about 50 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides in length having about 3 to about 25 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 25 to about 35 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35) nucleotides that is chemically-modified with one or more chemical modifications having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 3 to about 25 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs and a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV). In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. In one embodiment, a linear hairpin siNA molecule of the invention comprises a loop portion comprising a non-nucleotide linker.

In another embodiment, a siNA molecule of the invention comprises an asymmetric hairpin structure, wherein the siNA is about 25 to about 50 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides in length having about 3 to about 25 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 25 to about 35 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35) nucleotides

that is chemically-modified with one or more chemical modifications having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms an asymmetric hairpin structure having about 3 to about 25 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs and a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV). In one embodiment, an asymmetric hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. In another embodiment, an asymmetric hairpin siNA molecule of the invention comprises a loop portion comprising a non-nucleotide linker.

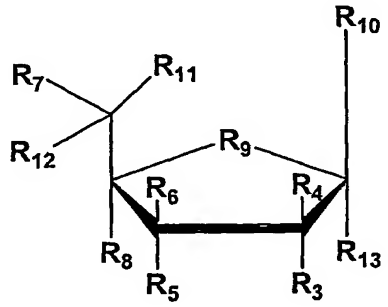
In another embodiment, a siNA molecule of the invention comprises an asymmetric double stranded structure having separate polynucleotide strands comprising sense and antisense regions, wherein the antisense region is about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length, wherein the sense region is about 3 to about 25 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides in length, wherein the sense region and the antisense region have at least 3 complementary nucleotides, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises an asymmetric double stranded structure having separate polynucleotide strands comprising sense and antisense regions, wherein the antisense region is about 18 to about 23 (*e.g.*, about 18, 19, 20, 21, 22, or 23) nucleotides in length and wherein the sense region is about 3 to about 15 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) nucleotides in length, wherein the sense region the antisense region have at least 3 complementary nucleotides, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. In another embodiment, the asymmetic double stranded siNA molecule can also have a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV).

In another embodiment, a siNA molecule of the invention comprises a circular nucleic acid molecule, wherein the siNA is about 38 to about 70 (*e.g.*, about 38, 40, 45,

50, 55, 60, 65, or 70) nucleotides in length having about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein the siNA can include a chemical modification, which comprises a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a circular oligonucleotide having about 42 to about 50 (*e.g.*, about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the circular oligonucleotide forms a dumbbell shaped structure having about 19 base pairs and 2 loops.

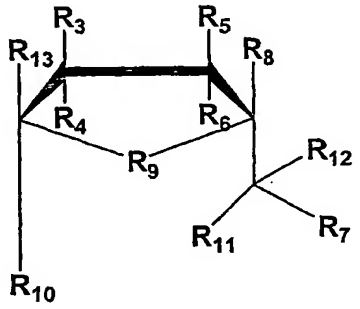
In another embodiment, a circular siNA molecule of the invention contains two loop motifs, wherein one or both loop portions of the siNA molecule is biodegradable. For example, a circular siNA molecule of the invention is designed such that degradation of the loop portions of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In one embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) abasic moiety, for example a compound having Formula V:



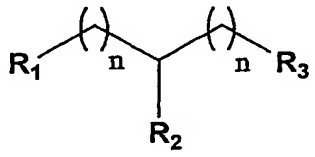
wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2.

In one embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) inverted abasic moiety, for example a compound having Formula VI:



wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2, and either R2, R3, R8 or R13 serve as points of attachment to the siNA molecule of the invention.

In another embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) substituted polyalkyl moieties, for example a compound having Formula VII:



wherein each n is independently an integer from 1 to 12, each R1, R2 and R3 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl,

aminoalkylamino, polyalklylamino, substituted silyl, or a group having Formula I, and R1, R2 or R3 serves as points of attachment to the siNA molecule of the invention.

In another embodiment, the invention features a compound having Formula VII, wherein R1 and R2 are hydroxyl (OH) groups, n = 1, and R3 comprises O and is the point of attachment to the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both strands of a double-stranded siNA molecule of the invention or to a single-stranded siNA molecule of the invention. This modification is referred to herein as "glyceryl" (for example modification 6 in **Figure 10**).

In another embodiment, a chemically modified nucleoside or non-nucleoside (e.g. a moiety having any of Formula V, VI or VII) of the invention is at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of a siNA molecule of the invention. For example, chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) can be present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense strand, the sense strand, or both antisense and sense strands of the siNA molecule. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the terminal position of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the two terminal positions of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the penultimate position of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In addition, a moiety having Formula VII can be present at the 3'-end or the 5'-end of a hairpin siNA molecule as described herein.

In another embodiment, a siNA molecule of the invention comprises an abasic residue having Formula V or VI, wherein the abasic residue having Formula VI or VI is

connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, a siNA molecule of the invention comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) locked nucleic acid (LNA) nucleotides, for example, at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In another embodiment, a siNA molecule of the invention comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) acyclic nucleotides, for example, at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), wherein any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein

any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said antisense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) against XIAP inside a cell or reconstituted *in vitro* system comprising a sense region, wherein one or more pyrimidine nucleotides present in the sense region are 2'-

deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and one or more purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), and an antisense region, wherein one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and one or more purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). The sense region and/or the antisense region can have a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense and/or antisense sequence. The sense and/or antisense region can optionally further comprise a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxynucleotides. The overhang nucleotides can further comprise one or more (e.g., about 1, 2, 3, 4 or more) phosphorothioate, phosphonoacetate, and/or thiophosphonoacetate internucleotide linkages. Non-limiting examples of these chemically-modified siNAs are shown in **Figures 4 and 5** and **Tables III and IV** herein. In any of these described embodiments, the purine nucleotides present in the sense region are alternatively 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides) and one or more purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). Also, in any of these embodiments, one or more purine nucleotides present in the sense region are alternatively purine ribonucleotides (e.g., wherein all purine nucleotides are purine ribonucleotides or alternately a plurality of purine nucleotides are purine ribonucleotides) and any purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a

plurality of purine nucleotides are 2'-O-methyl purine nucleotides). Additionally, in any of these embodiments, one or more purine nucleotides present in the sense region and/or present in the antisense region are alternatively selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides (e.g., wherein all purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides or alternately a plurality of purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides).

In another embodiment, any modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, are resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi. Non-limiting examples of nucleotides having a northern configuration include locked nucleic acid (LNA) nucleotides (e.g., 2′-O, 4′-C-methylene-(D-ribofuranosyl) nucleotides); 2'-methoxyethoxy (MOE) nucleotides; 2'-methyl-thio-ethyl, 2'-deoxy-2'-fluoro nucleotides, 2'-deoxy-2'-chloro nucleotides, 2'-azido nucleotides, and 2'-O-methyl nucleotides.

In one embodiment, the sense strand of a double stranded siNA molecule of the invention comprises a terminal cap moiety, (see for example **Figure 10**) such as an inverted deoxyabaisc moiety, at the 3'-end, 5'-end, or both 3' and 5'-ends of the sense strand.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid molecule (siNA) capable of mediating RNA interference (RNAi) against XIAP inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a conjugate covalently attached to the chemically-modified siNA molecule. Non-limiting examples of conjugates contemplated by the invention include conjugates and ligands described in Vargeese *et al.*, USSN 10/427,160, filed April 30, 2003, incorporated by reference herein in its entirety, including the drawings. In another embodiment, the conjugate is covalently attached to the chemically-modified siNA molecule via a biodegradable linker. In one embodiment, the conjugate molecule is attached at the 3'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In another embodiment, the conjugate molecule is attached at the 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In yet another embodiment, the conjugate molecule is attached both the 3'-end and 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule, or any combination thereof. In one embodiment, a conjugate molecule of the invention comprises a molecule that facilitates delivery of a chemically-modified siNA molecule into a biological system, such as a cell. In another embodiment, the conjugate molecule attached to the chemically-modified siNA molecule is a polyethylene glycol, human serum albumin, or a ligand for a cellular receptor that can mediate cellular uptake. Examples of specific conjugate molecules contemplated by the instant invention that can be attached to chemically-modified siNA molecules are described in Vargeese *et al.*, U.S. Serial No. 10/201,394, filed July 22, 2002 incorporated by reference herein. The type of conjugates used and the extent of conjugation of siNA molecules of the invention can be evaluated for improved pharmacokinetic profiles, bioavailability, and/or stability of siNA constructs while at the same time maintaining the ability of the siNA to mediate RNAi activity. As such, one skilled in the art can screen siNA constructs that are modified with various conjugates to determine whether the siNA conjugate complex possesses improved properties while maintaining the ability to mediate RNAi, for example in animal models as are generally known in the art.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule of the invention, wherein the siNA further comprises a nucleotide, non-

nucleotide, or mixed nucleotide/non-nucleotide linker that joins the sense region of the siNA to the antisense region of the siNA. In one embodiment, a nucleotide linker of the invention can be a linker of ≥ 2 nucleotides in length, for example about 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length. In another embodiment, the nucleotide linker can be a nucleic acid aptamer. By "aptamer" or "nucleic acid aptamer" as used herein is meant a nucleic acid molecule that binds specifically to a target molecule wherein the nucleic acid molecule has sequence that comprises a sequence recognized by the target molecule in its natural setting. Alternately, an aptamer can be a nucleic acid molecule that binds to a target molecule where the target molecule does not naturally bind to a nucleic acid. The target molecule can be any molecule of interest. For example, the aptamer can be used to bind to a ligand-binding domain of a protein, thereby preventing interaction of the naturally occurring ligand with the protein. This is a non-limiting example and those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. (See, for example, Gold *et al.*, 1995, *Annu. Rev. Biochem.*, 64, 763; Brody and Gold, 2000, *J. Biotechnol.*, 74, 5; Sun, 2000, *Curr. Opin. Mol. Ther.*, 2, 100; Kusser, 2000, *J. Biotechnol.*, 74, 27; Hermann and Patel, 2000, *Science*, 287, 820; and Jayasena, 1999, *Clinical Chemistry*, 45, 1628.)

In yet another embodiment, a non-nucleotide linker of the invention comprises abasic nucleotide, polyether, polyamine, polyamide, peptide, carbohydrate, lipid, polyhydrocarbon, or other polymeric compounds (e.g. polyethylene glycols such as those having between 2 and 100 ethylene glycol units). Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* 1990, *18*:6353 and *Nucleic Acids Res.* 1987, *15*:3113; Cload and Schepartz, *J. Am. Chem. Soc.* 1991, *113*:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* 1991, *113*:5109; Ma *et al.*, *Nucleic Acids Res.* 1993, *21*:2585 and *Biochemistry* 1993, *32*:1751; Durand *et al.*, *Nucleic Acids Res.* 1990, *18*:6353; McCurdy *et al.*, *Nucleosides & Nucleotides* 1991, *10*:287; Jschke *et al.*, *Tetrahedron Lett.* 1993, *34*:301; Ono *et al.*, *Biochemistry* 1991, *30*:9914; Arnold *et al.*, International Publication No. WO 89/02439; Usman *et al.*, International Publication No. WO 95/06731; Dudycz *et al.*, International Publication No. WO 95/11910 and Ferentz and Verdine, *J. Am. Chem. Soc.* 1991, *113*:4000, all hereby incorporated by reference herein. A "non-nucleotide" further means any group or compound that can be incorporated into a nucleic acid chain in the place of one or more nucleotide units,

including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound can be abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine, for example at the C1 position of the sugar.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted *in vitro* system, wherein one or both strands of the siNA molecule that are assembled from two separate oligonucleotides do not comprise any ribonucleotides. For example, a siNA molecule can be assembled from a single oligonculeotide where the sense and antisense regions of the siNA comprise separate oligonucleotides that do not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotides. In another example, a siNA molecule can be assembled from a single oligonculeotide where the sense and antisense regions of the siNA are linked or circularized by a nucleotide or non-nucleotide linker as described herein, wherein the oligonucleotide does not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotide. Applicant has surprisingly found that the presense of ribonucleotides (e.g., nucleotides having a 2'-hydroxyl group) within the siNA molecule is not required or essential to support RNAi activity. As such, in one embodiment, all positions within the siNA can include chemically modified nucleotides and/or non-nucleotides such as nucleotides and or non-nucleotides having Formula I, II, III, IV, V, VI, or VII or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted *in vitro* system comprising a single stranded polynucleotide having complementarity to a target nucleic acid sequence. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group and a 3'-terminal phosphate group (e.g., a 2',3'-cyclic phosphate). In another embodiment, the single stranded siNA molecule of the invention comprises about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides. In yet another embodiment, the single stranded siNA molecule of the invention comprises one

or more chemically modified nucleotides or non-nucleotides described herein. For example, all the positions within the siNA molecule can include chemically-modified nucleotides such as nucleotides having any of Formulae I-VII, or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted *in vitro* system comprising a single stranded polynucleotide having complementarity to a target nucleic acid sequence, wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence. The siNA optionally further comprises about 1 to about 4 or more (e.g., about 1, 2, 3, 4 or more) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, 4 or more) phosphorothioate, phosphonoacetate, and/or thiophosphonoacetate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group. In any of these embodiments, any purine nucleotides present in the antisense region are alternatively 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides). Also, in any of these embodiments, any purine nucleotides present in the siNA (i.e., purine nucleotides present in the sense and/or antisense region) can alternatively be locked nucleic acid (LNA) nucleotides (e.g., wherein all purine nucleotides are LNA nucleotides or alternately a plurality of purine nucleotides are LNA nucleotides). Also, in any of these embodiments, any purine nucleotides present in the siNA are alternatively 2'-methoxyethyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-

methoxyethyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-methoxyethyl purine nucleotides). In another embodiment, any modified nucleotides present in the single stranded siNA molecules of the invention comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the single stranded siNA molecules of the invention are preferably resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi.

In one embodiment, a siNA molecule of the invention comprises chemically modified nucleotides or non-nucleotides (e.g., having any of Formulae I-VII, such as 2'-deoxy, 2'-deoxy-2'-fluoro, or 2'-O-methyl nucleotides) at alternating positions within one or more strands or regions of the siNA molecule. For example, such chemical modifications can be introduced at every other position of a RNA based siNA molecule, starting at either the first or second nucleotide from the 3'-end or 5'-end of the siNA. In a non-limiting example, a double stranded siNA molecule of the invention in which each strand of the siNA is 21 nucleotides in length is featured wherein positions 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21 of each strand are chemically modified (e.g., with compounds having any of Formulae 1-VII, such as such as 2'-deoxy, 2'-deoxy-2'-fluoro, or 2'-O-methyl nucleotides). In another non-limiting example, a double stranded siNA molecule of the invention in which each strand of the siNA is 21 nucleotides in length is featured wherein positions 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 of each strand are chemically modified (e.g., with compounds having any of Formulae 1-VII, such as such as 2'-deoxy, 2'-deoxy-2'-fluoro, or 2'-O-methyl nucleotides). Such siNA molecules can further comprise terminal cap moieties and/or backbone modifications as described herein.

In one embodiment, the invention features a method for modulating the expression of a XIAP gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the XIAP gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the XIAP gene in the cell.

In one embodiment, the invention features a method for modulating the expression of a XIAP gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the XIAP gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequence of the target RNA; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the XIAP gene in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one XIAP gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the XIAP genes; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate the expression of the XIAP genes in the cell.

In another embodiment, the invention features a method for modulating the expression of two or more XIAP genes within a cell comprising: (a) synthesizing one or more siNA molecules of the invention, which can be chemically-modified, wherein the siNA strands comprise sequences complementary to RNA of the XIAP genes and wherein the sense strand sequences of the siNAs comprise sequences identical or substantially similar to the sequences of the target RNAs; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate the expression of the XIAP genes in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one XIAP gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the XIAP gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequences of the target RNAs; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the XIAP genes in the cell.

In one embodiment, siNA molecules of the invention are used as reagents in *ex vivo* applications. For example, siNA reagents are introduced into tissue or cells that are

transplanted into a subject for therapeutic effect. The cells and/or tissue can be derived from an organism or subject that later receives the explant, or can be derived from another organism or subject prior to transplantation. The siNA molecules can be used to modulate the expression of one or more genes in the cells or tissue, such that the cells or tissue obtain a desired phenotype or are able to perform a function when transplanted in vivo. In one embodiment, certain target cells from a patient are extracted. These extracted cells are contacted with siNAs targeting a specific nucleotide sequence within the cells under conditions suitable for uptake of the siNAs by these cells (e.g. using delivery reagents such as cationic lipids, liposomes and the like or using techniques such as electroporation to facilitate the delivery of siNAs into cells). The cells are then reintroduced back into the same patient or other patients. In one embodiment, the invention features a method of modulating the expression of a XIAP gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the XIAP gene; and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the XIAP gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the XIAP gene in that organism.

In one embodiment, the invention features a method of modulating the expression of a XIAP gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the XIAP gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequence of the target RNA; and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the XIAP gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the XIAP gene in that organism.

In another embodiment, the invention features a method of modulating the expression of more than one XIAP gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the XIAP genes; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the XIAP genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the XIAP genes in that organism.

In one embodiment, the invention features a method of modulating the expression of a XIAP gene in a subject or organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the XIAP gene; and (b) introducing the siNA molecule into the subject or organism under conditions suitable to modulate the expression of the XIAP gene in the subject or organism. The level of XIAP protein or RNA can be determined using various methods well-known in the art.

In another embodiment, the invention features a method of modulating the expression of more than one XIAP gene in a subject or organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the XIAP genes; and (b) introducing the siNA molecules into the subject or organism under conditions suitable to modulate the expression of the XIAP genes in the subject or organism. The level of XIAP protein or RNA can be determined as is known in the art.

In one embodiment, the invention features a method for modulating the expression of a XIAP gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the XIAP gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the XIAP gene in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one XIAP gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the XIAP gene; and (b) contacting the cell *in vitro* or *in vivo* with the siNA molecule under conditions suitable to modulate the expression of the XIAP genes in the cell.

In one embodiment, the invention features a method of modulating the expression of a XIAP gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the XIAP gene; and (b) contacting a cell of the tissue explant derived from a particular subject or organism with the siNA molecule under conditions suitable to modulate the expression of the XIAP gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the subject or organism the tissue was derived from or into another subject or organism under conditions suitable to modulate the expression of the XIAP gene in that subject or organism.

In another embodiment, the invention features a method of modulating the expression of more than one XIAP gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the XIAP gene; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular subject or organism under conditions suitable to modulate the expression of the XIAP genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the subject or organism the tissue was derived from or into another subject or organism under conditions suitable to modulate the expression of the XIAP genes in that subject or organism.

In one embodiment, the invention features a method of modulating the expression of a XIAP gene in a subject or organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the XIAP gene; and (b)

introducing the siNA molecule into the subject or organism under conditions suitable to modulate the expression of the XIAP gene in the subject or organism.

In another embodiment, the invention features a method of modulating the expression of more than one XIAP gene in a subject or organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the XIAP gene; and (b) introducing the siNA molecules into the subject or organism under conditions suitable to modulate the expression of the XIAP genes in the subject or organism.

In one embodiment, the invention features a method of modulating the expression of a XIAP gene in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the XIAP gene in the subject or organism.

In one embodiment, the invention features a method for treating or preventing cancer, such as ovarian cancer; cancers of non-lymphoid parenchymal organs including the heart, placenta, skeletal muscle and lung; breast cancer; cancers of the head and neck, including various lymphomas such as mantle cell lymphoma; non-Hodgkins lymphoma; adenoma; squamous cell carcinoma; laryngeal carcinoma; cancers of the retina; cancers of the esophagus; multiple myeloma; melanoma; colorectal cancer; lung cancer; bladder cancer; prostate cancer; and glioblastoma comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of XIAP gene in the subject or organism.

In one embodiment, the invention features a method for treating or preventing proliferative disorders, such as restenosis, comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the XIAP gene in the subject or organism.

In one embodiment, the invention features a method for treating or preventing polycystic kidney disease in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the XIAP gene in the subject or organism.

In one embodiment, the invention features a method for treating or preventing ocular disease in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the XIAP gene in the subject or organism.

In another embodiment, the invention features a method of modulating the expression of more than one XIAP genes in a subject or organism comprising contacting the subject or organism with one or more siNA molecules of the invention under conditions suitable to modulate the expression of the XIAP genes in the subject or organism.

The siNA molecules of the invention can be designed to down regulate or inhibit target (e.g., XIAP) gene expression through RNAi targeting of a variety of RNA molecules. In one embodiment, the siNA molecules of the invention are used to target various RNAs corresponding to a target gene. Non-limiting examples of such RNAs include messenger RNA (mRNA), alternate RNA splice variants of target gene(s), post-transcriptionally modified RNA of target gene(s), pre-mRNA of target gene(s), and/or RNA templates. If alternate splicing produces a family of transcripts that are distinguished by usage of appropriate exons, the instant invention can be used to inhibit gene expression through the appropriate exons to specifically inhibit or to distinguish among the functions of gene family members. For example, a protein that contains an alternatively spliced transmembrane domain can be expressed in both membrane bound and secreted forms. Use of the invention to target the exon containing the transmembrane domain can be used to determine the functional consequences of pharmaceutical targeting of membrane bound as opposed to the secreted form of the protein. Non-limiting examples of applications of the invention relating to targeting these RNA molecules include therapeutic pharmaceutical applications, pharmaceutical discovery applications, molecular diagnostic and gene function applications, and gene mapping, for example using single nucleotide polymorphism mapping with siNA molecules of the invention. Such applications can be implemented using known gene sequences or from partial sequences available from an expressed sequence tag (EST).

In another embodiment, the siNA molecules of the invention are used to target conserved sequences corresponding to a gene family or gene families such as XIAP

family genes. As such, siNA molecules targeting multiple XIAP targets can provide increased therapeutic effect. In addition, siNA can be used to characterize pathways of gene function in a variety of applications. For example, the present invention can be used to inhibit the activity of target gene(s) in a pathway to determine the function of uncharacterized gene(s) in gene function analysis, mRNA function analysis, or translational analysis. The invention can be used to determine potential target gene pathways involved in various diseases and conditions toward pharmaceutical development. The invention can be used to understand pathways of gene expression involved in, for example, cancer and other proliferative disorders.

In one embodiment, siNA molecule(s) and/or methods of the invention are used to down regulate the expression of gene(s) that encode RNA referred to by Genbank Accession, for example, XIAP genes encoding RNA sequence(s) referred to herein by Genbank Accession number, for example, Genbank Accession Nos. shown in **Table I.**

In one embodiment, the invention features a method comprising: (a) generating a library of siNA constructs having a predetermined complexity; and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target RNA sequence. In one embodiment, the siNA molecules of (a) have strands of a fixed length, for example, about 23 nucleotides in length. In another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In one embodiment, the invention features a method comprising: (a) generating a randomized library of siNA constructs having a predetermined complexity, such as of 4^N, where N represents the number of base paired nucleotides in each of the siNA construct

strands (*eg.* for a siNA construct having 21 nucleotide sense and antisense strands with 19 base pairs, the complexity would be 4^{19}); and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target XIAP RNA sequence. In another embodiment, the siNA molecules of (a) have strands of a fixed length, for example about 23 nucleotides in length. In yet another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described in Example 6 herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of XIAP RNA are analyzed for detectable levels of cleavage, for example, by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target XIAP RNA sequence. The target XIAP RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In another embodiment, the invention features a method comprising: (a) analyzing the sequence of a RNA target encoded by a target gene; (b) synthesizing one or more sets of siNA molecules having sequence complementary to one or more regions of the RNA of (a); and (c) assaying the siNA molecules of (b) under conditions suitable to determine RNAi targets within the target RNA sequence. In one embodiment, the siNA molecules of (b) have strands of a fixed length, for example about 23 nucleotides in length. In another embodiment, the siNA molecules of (b) are of differing length, for example having strands of about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. Fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by expression in *in vivo* systems.

By "target site" is meant a sequence within a target RNA that is "targeted" for cleavage mediated by a siNA construct which contains sequences within its antisense region that are complementary to the target sequence.

By "detectable level of cleavage" is meant cleavage of target RNA (and formation of cleaved product RNAs) to an extent sufficient to discern cleavage products above the background of RNAs produced by random degradation of the target RNA. Production of cleavage products from 1-5% of the target RNA is sufficient to detect above the background for most methods of detection.

In one embodiment, the invention features a composition comprising a siNA molecule of the invention, which can be chemically-modified, in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a pharmaceutical composition comprising siNA molecules of the invention, which can be chemically-modified, targeting one or more genes in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a method for diagnosing a disease or condition in a subject comprising administering to the subject a composition of the invention under conditions suitable for the diagnosis of the disease or condition in the subject. In another embodiment, the invention features a method for treating or preventing a disease or condition in a subject, comprising administering to the subject a composition of the invention under conditions suitable for the treatment or prevention of the disease or condition in the subject, alone or in conjunction with one or more other therapeutic compounds.

In another embodiment, the invention features a method for validating a XIAP gene target, comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a XIAP target gene; (b) introducing the siNA molecule into a cell, tissue, subject, or organism under conditions suitable for modulating expression of the XIAP target gene in the cell, tissue, subject, or organism; and (c) determining the function of the gene by assaying for any phenotypic change in the cell, tissue, subject, or organism.

In another embodiment, the invention features a method for validating a XIAP target comprising: (a) synthesizing a siNA molecule of the invention, which can be

chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a XIAP target gene; (b) introducing the siNA molecule into a biological system under conditions suitable for modulating expression of the XIAP target gene in the biological system; and (c) determining the function of the gene by assaying for any phenotypic change in the biological system.

By "biological system" is meant, material, in a purified or unpurified form, from biological sources, including but not limited to human or animal, wherein the system comprises the components required for RNAi activity. The term "biological system" includes, for example, a cell, tissue, subject, or organism, or extract thereof. The term biological system also includes reconstituted RNAi systems that can be used in an *in vitro* setting.

By "phenotypic change" is meant any detectable change to a cell that occurs in response to contact or treatment with a nucleic acid molecule of the invention (e.g., siNA). Such detectable changes include, but are not limited to, changes in shape, size, proliferation, motility, protein expression or RNA expression or other physical or chemical changes as can be assayed by methods known in the art. The detectable change can also include expression of reporter genes/molecules such as Green Florescent Protein (GFP) or various tags that are used to identify an expressed protein or any other cellular component that can be assayed.

In one embodiment, the invention features a kit containing a siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of a XIAP target gene in a biological system, including, for example, in a cell, tissue, subject, or organism. In another embodiment, the invention features a kit containing more than one siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of more than one XIAP target gene in a biological system, including, for example, in a cell, tissue, subject, or organism.

In one embodiment, the invention features a cell containing one or more siNA molecules of the invention, which can be chemically-modified. In another embodiment, the cell containing a siNA molecule of the invention is a mammalian cell. In yet another embodiment, the cell containing a siNA molecule of the invention is a human cell.

In one embodiment, the synthesis of a siNA molecule of the invention, which can be chemically-modified, comprises: (a) synthesis of two complementary strands of the siNA molecule; (b) annealing the two complementary strands together under conditions suitable to obtain a double-stranded siNA molecule. In another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase oligonucleotide synthesis. In yet another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase tandem oligonucleotide synthesis.

In one embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing a first oligonucleotide sequence strand of the siNA molecule, wherein the first oligonucleotide sequence strand comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of the second oligonucleotide sequence strand of the siNA; (b) synthesizing the second oligonucleotide sequence strand of siNA on the scaffold of the first oligonucleotide sequence strand, wherein the second oligonucleotide sequence strand further comprises a chemical moiety than can be used to purify the siNA duplex; (c) cleaving the linker molecule of (a) under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex; and (d) purifying the siNA duplex utilizing the chemical moiety of the second oligonucleotide sequence strand. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example, under hydrolysis conditions using an alkylamine base such as methylamine. In one embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place concomitantly. In another embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group, which can be employed in a trityl-on synthesis strategy as described herein. In yet another embodiment, the chemical moiety, such as a dimethoxytrityl group, is removed during purification, for example, using acidic conditions.

In a further embodiment, the method for siNA synthesis is a solution phase synthesis or hybrid phase synthesis wherein both strands of the siNA duplex are synthesized in tandem using a cleavable linker attached to the first sequence which acts a scaffold for synthesis of the second sequence. Cleavage of the linker under conditions suitable for hybridization of the separate siNA sequence strands results in formation of the double-stranded siNA molecule.

In another embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing one oligonucleotide sequence strand of the siNA molecule, wherein the sequence comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of another oligonucleotide sequence; (b) synthesizing a second oligonucleotide sequence having complementarity to the first sequence strand on the scaffold of (a), wherein the second sequence comprises the other strand of the double-stranded siNA molecule and wherein the second sequence further comprises a chemical moiety than can be used to isolate the attached oligonucleotide sequence; (c) purifying the product of (b) utilizing the chemical moiety of the second oligonucleotide sequence strand under conditions suitable for isolating the full-length sequence comprising both siNA oligonucleotide strands connected by the cleavable linker and under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example, under hydrolysis conditions. In another embodiment, cleavage of the linker molecule in (c) above takes place after deprotection of the oligonucleotide. In another embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity or differing reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place either concomitantly or sequentially. In one embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group.

In another embodiment, the invention features a method for making a double-stranded siNA molecule in a single synthetic process comprising: (a) synthesizing an oligonucleotide having a first and a second sequence, wherein the first sequence is complementary to the second sequence, and the first oligonucleotide sequence is linked to the second sequence via a cleavable linker, and wherein a terminal 5'-protecting group, for example, a 5'-O-dimethoxytrityl group (5'-O-DMT) remains on the oligonucleotide having the second sequence; (b) deprotecting the oligonucleotide whereby the deprotection results in the cleavage of the linker joining the two oligonucleotide sequences; and (c) purifying the product of (b) under conditions suitable for isolating the double-stranded siNA molecule, for example using a trityl-on synthesis strategy as described herein.

In another embodiment, the method of synthesis of siNA molecules of the invention comprises the teachings of Scaringe *et al.*, US Patent Nos. 5,889,136; 6,008,400; and 6,111,086, incorporated by reference herein in their entirety.

In one embodiment, the invention features siNA constructs that mediate RNAi against XIAP, wherein the siNA construct comprises one or more chemical modifications, for example, one or more chemical modifications having any of Formulae I-VII or any combination thereof that increases the nuclease resistance of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules with increased nuclease resistance comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased nuclease resistance.

In another embodiment, the invention features a method for generating siNA molecules with improved toxicologic profiles (e.g., have attenuated or no immunostimulatory properties) comprising (a) introducing nucleotides having any of Formula I-VII (e.g. siNA motifs referred to in Table IV) or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved toxicologic profiles.

In another embodiment, the invention features a method for generating siNA molecules that do not stimulate an interferon response (e.g., no interferon response or attenuated interferon response) in a cell, subject, or organism, comprising (a) introducing nucleotides having any of Formula I-VII (e.g. siNA motifs referred to in Table IV) or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules that do not stimulate an interferon response.

By "improved toxicological profile", is meant, that the chemically modified siNA construct exhibits decreased toxicity in a cell, subject, or organism compared to an unmodified siNA or siNA molecule having fewer modifications or modifications that are less effective in imparting improved toxicology. In a non-limiting example, siNA molecules with improved toxicologic profiles are associated with a decreased or attenuated immunostimulatory response in a cell, subject, or organism compared to an unmodified siNA or siNA molecule having fewer modifications or modifications that are less effective in imparting improved toxicology. In one embodiment, a siNA molecule with an improved toxicological profile comprises no ribonucleotides. In one embodiment, a siNA molecule with an improved toxicological profile comprises less than 5 ribonucleotides (e.g., 1, 2, 3, or 4 ribonucleotides). In one embodiment, a siNA molecule with an improved toxicological profile comprises Stab 7, Stab 8, Stab 11, Stab 12, Stab 13, Stab 16, Stab 17, Stab 18, Stab 19, Stab 20, Stab 23, Stab 24, Stab 25, Stab 26, Stab 27, Stab 28 or any combination thereof (see Table IV). In one embodiment, the level of immunostimulatory response associated with a given siNA molecule can be measured as is known in the art, for example by determining the level of PKR/interferon response, proliferation, B-cell activation, and/or cytokine production in assays to quantitate the immunostimulatory response of particular siNA molecules (see for example Leifer et al., 2003, J Immunother. 26, 313-9 and U.S. Patent No. 5,968,909, incorporated by reference herein).

In one embodiment, the invention features siNA constructs that mediate RNAi against XIAP, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the sense and antisense strands of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the sense and antisense strands of the siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the sense and antisense strands of the siNA molecule.

In one embodiment, the invention features siNA constructs that mediate RNAi against XIAP, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target RNA sequence within a cell.

In one embodiment, the invention features siNA constructs that mediate RNAi against XIAP, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target DNA sequence within a cell.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence.

In one embodiment, the invention features siNA constructs that mediate RNAi against XIAP, wherein the siNA construct comprises one or more chemical

modifications described herein that modulate the polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA construct.

In another embodiment, the invention features a method for generating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to a chemically-modified siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA molecule.

In one embodiment, the invention features chemically-modified siNA constructs that mediate RNAi against XIAP in a cell, wherein the chemical modifications do not significantly effect the interaction of siNA with a target RNA molecule, DNA molecule and/or proteins or other factors that are essential for RNAi in a manner that would decrease the efficacy of RNAi mediated by such siNA constructs.

In another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against XIAP comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity.

In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against XIAP target RNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target RNA.

In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against XIAP target DNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a

siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target DNA.

In one embodiment, the invention features siNA constructs that mediate RNAi against XIAP, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the cellular uptake of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules against XIAP with improved cellular uptake comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved cellular uptake.

In one embodiment, the invention features siNA constructs that mediate RNAi against XIAP, wherein the siNA construct comprises one or more chemical modifications described herein that increases the bioavailability of the siNA construct, for example, by attaching polymeric conjugates such as polyethyleneglycol or equivalent conjugates that improve the pharmacokinetics of the siNA construct, or by attaching conjugates that target specific tissue types or cell types *in vivo*. Non-limiting examples of such conjugates are described in Vargeese *et al.*, U.S. Serial No. 10/201,394 incorporated by reference herein.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing a conjugate into the structure of a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such conjugates can include ligands for cellular receptors, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and other co-factors, such as folate and N-acetylgalactosamine; polymers, such as polyethyleneglycol (PEG); phospholipids; cholesterol; polyamines, such as spermine or spermidine; and others.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary

to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence is chemically modified in a manner that it can no longer act as a guide sequence for efficiently mediating RNA interference and/or be recognized by cellular proteins that facilitate RNAi.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein the second sequence is designed or modified in a manner that prevents its entry into the RNAi pathway as a guide sequence or as a sequence that is complementary to a target nucleic acid (e.g., RNA) sequence. Such design or modifications are expected to enhance the activity of siNA and/or improve the specificity of siNA molecules of the invention. These modifications are also expected to minimize any off-target effects and/or associated toxicity.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence is incapable of acting as a guide sequence for mediating RNA interference.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence does not have a terminal 5'-hydroxyl (5'-OH) or 5'-phosphate group.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence comprises a terminal cap moiety at the 5'-end of said second sequence. In one embodiment, the terminal cap moiety comprises an inverted abasic, inverted deoxy abasic, inverted nucleotide moiety, a group shown in **Figure 10**, an alkyl or cycloalkyl group, a

heterocycle, or any other group that prevents RNAi activity in which the second sequence serves as a guide sequence or template for RNAi.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence comprises a terminal cap moiety at the 5'-end and 3'-end of said second sequence. In one embodiment, each terminal cap moiety individually comprises an inverted abasic, inverted deoxy abasic, inverted nucleotide moiety, a group shown in **Figure 10**, an alkyl or cycloalkyl group, a heterocycle, or any other group that prevents RNAi activity in which the second sequence serves as a guide sequence or template for RNAi.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved specificity for down regulating or inhibiting the expression of a target nucleic acid (e.g., a DNA or RNA such as a gene or its corresponding RNA), comprising (a) introducing one or more chemical modifications into the structure of a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved specificity. In another embodiment, the chemical modification used to improve specificity comprises terminal cap modifications at the 5'-end, 3'-end, or both 5' and 3'-ends of the siNA molecule. The terminal cap modifications can comprise, for example, structures shown in **Figure 10** (e.g. inverted deoxyabasic moieties) or any other chemical modification that renders a portion of the siNA molecule (e.g. the sense strand) incapable of mediating RNA interference against an off target nucleic acid sequence. In a non-limiting example, a siNA molecule is designed such that only the antisense sequence of the siNA molecule can serve as a guide sequence for RISC mediated degradation of a corresponding target RNA sequence. This can be accomplished by rendering the sense sequence of the siNA inactive by introducing chemical modifications to the sense strand that preclude recognition of the sense strand as a guide sequence by RNAi machinery. In one embodiment, such chemical modifications comprise any chemical group at the 5'-end of the sense strand of the siNA, or any other group that serves to render the sense strand inactive as a guide sequence for mediating RNA interference. These modifications, for example, can result in a molecule where the 5'-end of the sense strand no longer has a

free 5'-hydroxyl (5'-OH) or a free 5'-phosphate group (e.g., phosphate, diphosphate, triphosphate, cyclic phosphate etc.). Non-limiting examples of such siNA constructs are described herein, such as "Stab 9/10", "Stab 7/8", "Stab 7/19", "Stab 17/22", "Stab 23/24", "Stab 24/25" and "Stab 24/26" (e.g., any siNA having Stab 7, 9, 17, 23, or 24 sense strands) chemistries and variants thereof (see **Table IV**) wherein the 5'-end and 3'-end of the sense strand of the siNA do not comprise a hydroxyl group or phosphate group.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved specificity for down regulating or inhibiting the expression of a target nucleic acid (e.g., a DNA or RNA such as a gene or its corresponding RNA), comprising introducing one or more chemical modifications into the structure of a siNA molecule that prevent a strand or portion of the siNA molecule from acting as a template or guide sequence for RNAi activity. In one embodiment, the inactive strand or sense region of the siNA molecule is the sense strand or sense region of the siNA molecule, i.e. the strand or region of the siNA that does not have complementarity to the target nucleic acid sequence. In one embodiment, such chemical modifications comprise any chemical group at the 5'-end of the sense strand or region of the siNA that does not comprise a 5'-hydroxyl (5'-OH) or 5'-phosphate group, or any other group that serves to render the sense strand or sense region inactive as a guide sequence for mediating RNA interference. Non-limiting examples of such siNA constructs are described herein, such as "Stab 9/10", "Stab 7/8", "Stab 7/19", "Stab 17/22", "Stab 23/24", "Stab 24/25" and "Stab 24/26" (e.g., any siNA having Stab 7, 9, 17, 23, or 24 sense strands) chemistries and variants thereof (see **Table IV**) wherein the 5'-end and 3'-end of the sense strand of the siNA do not comprise a hydroxyl group or phosphate group.

In one embodiment, the invention features a method for screening siNA molecules that are active in mediating RNA interference against a target nucleic acid sequence comprising (a) generating a plurality of unmodified siNA molecules, (b) screening the siNA molecules of step (a) under conditions suitable for isolating siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence, and (c) introducing chemical modifications (e.g. chemical modifications as described herein or as otherwise known in the art) into the active siNA molecules of (b). In one

embodiment, the method further comprises re-screening the chemically modified siNA molecules of step (c) under conditions suitable for isolating chemically modified siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence.

In one embodiment, the invention features a method for screening chemically modified siNA molecules that are active in mediating RNA interference against a target nucleic acid sequence comprising (a) generating a plurality of chemically modified siNA molecules (e.g. siNA molecules as described herein or as otherwise known in the art), and (b) screening the siNA molecules of step (a) under conditions suitable for isolating chemically modified siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence.

The term "ligand" refers to any compound or molecule, such as a drug, peptide, hormone, or neurotransmitter, that is capable of interacting with another compound, such as a receptor, either directly or indirectly. The receptor that interacts with a ligand can be present on the surface of a cell or can alternately be an intercullular receptor. Interaction of the ligand with the receptor can result in a biochemical reaction, or can simply be a physical interaction or association.

In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing an excipient formulation to a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such excipients include polymers such as cyclodextrins, lipids, cationic lipids, polyamines, phospholipids, nanoparticles, receptors, ligands, and others.

In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing nucleotides having any of Formulae I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability.

In another embodiment, polyethylene glycol (PEG) can be covalently attached to siNA compounds of the present invention. The attached PEG can be any molecular weight, preferably from about 2,000 to about 50,000 daltons (Da).

The present invention can be used alone or as a component of a kit having at least one of the reagents necessary to carry out the *in vitro* or *in vivo* introduction of RNA to test samples and/or subjects. For example, preferred components of the kit include a siNA molecule of the invention and a vehicle that promotes introduction of the siNA into cells of interest as described herein (e.g., using lipids and other methods of transfection known in the art, see for example Beigelman *et al*, US 6,395,713). The kit can be used for target validation, such as in determining gene function and/or activity, or in drug optimization, and in drug discovery (see for example Usman et al., USSN 60/402,996). Such a kit can also include instructions to allow a user of the kit to practice the invention.

The term "short interfering nucleic acid", "siNA", "short interfering RNA", "siRNA", "short interfering nucleic acid molecule", "short interfering oligonucleotide molecule", or "chemically-modified short interfering nucleic acid molecule" as used herein refers to any nucleic acid molecule capable of inhibiting or down regulating gene expression or viral replication, for example by mediating RNA interference "RNAi" or gene silencing in a sequence-specific manner; see for example Zamore *et al.*, 2000, Cell, 101, 25-33; Bass, 2001, *Nature*, 411, 428-429; Elbashir *et al.*, 2001, *Nature*, 411, 494-498; and Kreutzer *et al.*, International PCT Publication No. WO 00/44895; Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck *et al.*, International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li *et al.*, International PCT Publication No. WO 00/44914; Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237; Hutvagner and Zamore, 2002, *Science*, 297, 2056-60; McManus *et al.*, 2002, *RNA*, 8, 842-850; Reinhart *et al.*, 2002, *Gene & Dev.*, 16, 1616-1626; and Reinhart & Bartel, 2002, *Science*, 297, 1831). Non limiting examples of siNA molecules of the invention are shown in **Figures 4-6**, and **Tables II and III** herein. For example the siNA can be a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions,

wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e. each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure, for example wherein the double stranded region is about 15 to about 30, *e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 base pairs; the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof (e.g., about 15 to about 25 or more nucleotides of the siNA molecule are complementary to the target nucleic acid or a portion thereof). Alternatively, the siNA is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the siNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s). The siNA can be a polynucleotide with a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either *in vivo* or *in vitro* to generate an active siNA molecule capable of mediating RNAi. The siNA can also comprise a single stranded polynucleotide having nucleotide sequence complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof (for example, where such siNA molecule does not require the presence within the siNA molecule of nucleotide

sequence corresponding to the target nucleic acid sequence or a portion thereof), wherein the single stranded polynucleotide can further comprise a terminal phosphate group, such as a 5'-phosphate (see for example Martinez *et al.*, 2002, *Cell.*, 110, 563-574 and Schwarz *et al.*, 2002, *Molecular Cell*, 10, 537-568), or 5',3'-diphosphate. In certain embodiments, the siNA molecule of the invention comprises separate sense and antisense sequences or regions, wherein the sense and antisense regions are covalently linked by nucleotide or non-nucleotide linkers molecules as is known in the art, or are alternately non-covalently linked by ionic interactions, hydrogen bonding, van der waals interactions, hydrophobic interactions, and/or stacking interactions. In certain embodiments, the siNA molecules of the invention comprise nucleotide sequence that is complementary to nucleotide sequence of a target gene. In another embodiment, the siNA molecule of the invention interacts with nucleotide sequence of a target gene in a manner that causes inhibition of expression of the target gene. As used herein, siNA molecules need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides. In certain embodiments, the short interfering nucleic acid molecules of the invention lack 2'-hydroxy (2'-OH) containing nucleotides. Applicant describes in certain embodiments short interfering nucleic acids that do not require the presence of nucleotides having a 2'-hydroxy group for mediating RNAi and as such, short interfering nucleic acid molecules of the invention optionally do not include any ribonucleotides (e.g., nucleotides having a 2'-OH group). Such siNA molecules that do not require the presence of ribonucleotides within the siNA molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. Optionally, siNA molecules can comprise ribonucleotides at about 5, 10, 20, 30, 40, or 50% of the nucleotide positions. The modified short interfering nucleic acid molecules of the invention can also be referred to as short interfering modified oligonucleotides "siMON." As used herein, the term siNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically-modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi

is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics. For example, siNA molecules of the invention can be used to epigenetically silence genes at both the post-transcriptional level or the pre-transcriptional level. In a non-limiting example, epigenetic regulation of gene expression by siNA molecules of the invention can result from siNA mediated modification of chromatin structure or methylation pattern to alter gene expression (see, for example, Verdel *et al.*, 2004, *Science,* 303, 672-676; Pal-Bhadra *et al.*, 2004, *Science,* 303, 669-672; Allshire, 2002, *Science,* 297, 1818-1819; Volpe *et al.*, 2002, *Science,* 297, 1833-1837; Jenuwein, 2002, *Science,* 297, 2215-2218; and Hall *et al.*, 2002, *Science,* 297, 2232-2237).

In one embodiment, a siNA molecule of the invention is a duplex forming oligonucleotide "DFO", (see for example **Figures 14-15** and Vaish et al., USSN 10/727,780 filed December 3, 2003 and International PCT Application No. US04/16390, filed May 24, 2004).

In one embodiment, a siNA molecule of the invention is a multifunctional siNA, (see for example **Figures 16-21** and Jadhav *et al.*, USSN 60/543,480 filed February 10, 2004 and International PCT Application No. US04/16390, filed May 24, 2004). The multifunctional siNA of the invention can comprise sequence targeting, for example, two regions of XIAP RNA (see for example target sequences in **Tables II and III**).

By "asymmetric hairpin" as used herein is meant a linear siNA molecule comprising an antisense region, a loop portion that can comprise nucleotides or non-nucleotides, and a sense region that comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complementary nucleotides to base pair with the antisense region and form a duplex with loop. For example, an asymmetric hairpin siNA molecule of the invention can comprise an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 15 to about 30, or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides) and a loop region comprising about 4 to about 12 (e.g., about 4, 5, 6, 7, 8, 9, 10, 11, or 12) nucleotides, and a sense region having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides that are complementary to the antisense region. The asymmetric hairpin siNA molecule can also

comprise a 5'-terminal phosphate group that can be chemically modified. The loop portion of the asymmetric hairpin siNA molecule can comprise nucleotides, non-nucleotides, linker molecules, or conjugate molecules as described herein.

By "asymmetric duplex" as used herein is meant a siNA molecule having two separate strands comprising a sense region and an antisense region, wherein the sense region comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complementary nucleotides to base pair with the antisense region and form a duplex. For example, an asymmetric duplex siNA molecule of the invention can comprise an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 15 to about 30, or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides) and a sense region having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides that are complementary to the antisense region.

By "modulate" is meant that the expression of the gene, or level of RNA molecule or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits is up regulated or down regulated, such that expression, level, or activity is greater than or less than that observed in the absence of the modulator. For example, the term "modulate" can mean "inhibit," but the use of the word "modulate" is not limited to this definition.

By "inhibit", "down-regulate", or "reduce", it is meant that the expression of the gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits, is reduced below that observed in the absence of the nucleic acid molecules (e.g., siNA) of the invention. In one embodiment, inhibition, down-regulation or reduction with an siNA molecule is below that level observed in the presence of an inactive or attenuated molecule. In another embodiment, inhibition, down-regulation, or reduction with siNA molecules is below that level observed in the presence of, for example, an siNA molecule with scrambled sequence or with mismatches. In another embodiment, inhibition, down-regulation, or reduction of gene expression with a nucleic acid molecule of the instant invention is greater in the presence of the nucleic acid molecule than in its absence. In one embodiment, inhibition, down regulation, or reduction of gene

expression is associated with post transcriptional silencing, such as RNAi mediated cleavage of a target nucleic acid molecule (e.g. RNA) or inhibition of translation. In one embodiment, inhibition, down regulation, or reduction of gene expression is associated with pretranscriptional silencing.

By "gene", or "target gene", is meant a nucleic acid that encodes an RNA, for example, nucleic acid sequences including, but not limited to, structural genes encoding a polypeptide. A gene or target gene can also encode a functional RNA (fRNA) or non-coding RNA (ncRNA), such as small temporal RNA (stRNA), micro RNA (miRNA), small nuclear RNA (snRNA), short interfering RNA (siRNA), small nucleolar RNA (snRNA), ribosomal RNA (rRNA), transfer RNA (tRNA) and precursor RNAs thereof. Such non-coding RNAs can serve as target nucleic acid molecules for siNA mediated RNA interference in modulating the activity of fRNA or ncRNA involved in functional or regulatory cellular processes. Abberant fRNA or ncRNA activity leading to disease can therefore be modulated by siNA molecules of the invention. siNA molecules targeting fRNA and ncRNA can also be used to manipulate or alter the genotype or phenotype of a subject, organism or cell, by intervening in cellular processes such as genetic imprinting, transcription, translation, or nucleic acid processing (e.g., transamination, methylation etc.). The target gene can be a gene derived from a cell, an endogenous gene, a transgene, or exogenous genes such as genes of a pathogen, for example a virus, which is present in the cell after infection thereof. The cell containing the target gene can be derived from or contained in any organism, for example a plant, animal, protozoan, virus, bacterium, or fungus. Non-limiting examples of plants include monocots, dicots, or gymnosperms. Non-limiting examples of animals include vertebrates or invertebrates. Non-limiting examples of fungi include molds or yeasts. For a review, see for example Snyder and Gerstein, 2003, *Science*, 300, 258-260.

By "non-canonical base pair" is meant any non-Watson Crick base pair, such as mismatches and/or wobble base pairs, inlcuding flipped mismatches, single hydrogen bond mismatches, trans-type mismatches, triple base interactions, and quadruple base interactions. Non-limiting examples of such non-canonical base pairs include, but are not limited to, AC reverse Hoogsteen, AC wobble, AU reverse Hoogsteen, GU wobble, AA N7 amino, CC 2-carbonyl-amino(H1)-N3-amino(H2), GA sheared, UC 4-carbonyl-amino, UU imino-carbonyl, AC reverse wobble, AU Hoogsteen, AU reverse Watson

Crick, CG reverse Watson Crick, GC N3-amino-amino N3, AA N1-amino symmetric, AA N7-amino symmetric, GA N7-N1 amino-carbonyl, GA+ carbonyl-amino N7-N1, GG N1-carbonyl symmetric, GG N3-amino symmetric, CC carbonyl-amino symmetric, CC N3-amino symmetric, UU 2-carbonyl-imino symmetric, UU 4-carbonyl-imino symmetric, AA amino-N3, AA N1-amino, AC amino 2-carbonyl, AC N3-amino, AC N7-amino, AU amino-4-carbonyl, AU N1-imino, AU N3-imino, AU N7-imino, CC carbonyl-amino, GA amino-N1, GA amino-N7, GA carbonyl-amino, GA N3-amino, GC amino-N3, GC carbonyl-amino, GC N3-amino, GC N7-amino, GG amino-N7, GG carbonyl-imino, GG N7-amino, GU amino-2-carbonyl, GU carbonyl-imino, GU imino-2-carbonyl, GU N7-imino, psiU imino-2-carbonyl, UC 4-carbonyl-amino, UC imino-carbonyl, UU imino-4-carbonyl, AC C2-H-N3, GA carbonyl-C2-H, UU imino-4-carbonyl 2 carbonyl-C5-H, AC amino(A) N3(C)-carbonyl, GC imino amino-carbonyl, Gpsi imino-2-carbonyl amino-2- carbonyl, and GU imino amino-2-carbonyl base pairs.

By "XIAP" as used herein is meant, any X-linked inhibitor of apoptosis (XIAP) protein, peptide, or polypeptide having XIAP activity, such as encoded by XIAP Genbank Accession Nos. shown in **Table I**. The term XIAP also refers to nucleic acid sequences encloding any XIAP protein, peptide, or polypeptide having XIAP activity. The term XIAP as used herein also refers to other inhibitor of apoptosis genes (XIAP) encoding inhibitor of apoptosis proteins, such as HIAP1, HIAP2, and/or NAIP. The term "XIAP" is also meant to include other XIAP encoding sequence, such as XIAP isoforms, mutant XIAP genes, splice variants of XIAP genes, and XIAP gene polymorphisms. By "homologous sequence" is meant, a nucleotide sequence that is shared by one or more polynucleotide sequences, such as genes, gene transcripts and/or non-coding polynucleotides. For example, a homologous sequence can be a nucleotide sequence that is shared by two or more genes encoding related but different proteins, such as different members of a gene family, different protein epitopes, different protein isoforms or completely divergent genes, such as a cytokine and its corresponding receptors. A homologous sequence can be a nucleotide sequence that is shared by two or more non-coding polynucleotides, such as noncoding DNA or RNA, regulatory sequences, introns, and sites of transcriptional control or regulation. Homologous sequences can also include conserved sequence regions shared by more than one polynucleotide sequence. Homology does not need to be perfect homology (e.g., 100%), as partially homologous

sequences are also contemplated by the instant invention (e.g., 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% etc.).

By "conserved sequence region" is meant, a nucleotide sequence of one or more regions in a polynucleotide does not vary significantly between generations or from one biological system, subject, or organism to another biological system, subject, or organism. The polynucleotide can include both coding and non-coding DNA and RNA.

By "sense region" is meant a nucleotide sequence of a siNA molecule having complementarity to an antisense region of the siNA molecule. In addition, the sense region of a siNA molecule can comprise a nucleic acid sequence having homology with a target nucleic acid sequence.

By "antisense region" is meant a nucleotide sequence of a siNA molecule having complementarity to a target nucleic acid sequence. In addition, the antisense region of a siNA molecule can optionally comprise a nucleic acid sequence having complementarity to a sense region of the siNA molecule.

By "target nucleic acid" is meant any nucleic acid sequence whose expression or activity is to be modulated. The target nucleic acid can be DNA or RNA.

By "complementarity" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity. Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner *et al.*, 1987, *CSH Symp. Quant. Biol.* LII pp.123-133; Frier *et al.*, 1986, *Proc. Nat. Acad. Sci.* USA 83:9373-9377; Turner *et al.*, 1987, *J. Am. Chem. Soc.* 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, or 10 nucleotides out of a total of 10 nucleotides in the first oligonucleotide being based paired to a second nucleic acid sequence having 10 nucleotides represents 50%, 60%, 70%, 80%, 90%, and

100% complementary respectively). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. In one embodiment, a siNA molecule of the invention comprises about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides that are complementary to one or more target nucleic acid molecules or a portion thereof.

In one embodiment, siNA molecules of the invention that down regulate or reduce XIAP gene expression are used for preventing or treating, in a subject or organism, a variety of oncogenic and proliferative diseases and disorders. By "proliferative disease" or "cancer" as used herein is meant, any disease or condition characterized by unregulated cell growth or replication as is known in the art, including various cancers including but not limited to multiple drug resistant cancers, such as leukemias including acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), Acute lymphocytic leukemia (ALL), and chronic lymphocytic leukemia; AIDS related cancers such as Kaposi's sarcoma; breast cancers; bone cancers such as Osteosarcoma, Chondrosarcomas, Ewing's sarcoma, Fibrosarcomas, Giant cell tumors, Adamantinomas, and Chordomas; Brain cancers such as Meningiomas, Glioblastomas, Lower-Grade Astrocytomas, Oligodendrocytomas, Pituitary Tumors, Schwannomas, and Metastatic brain cancers; cancers of the head and neck including various lymphomas such as mantle cell lymphoma, non-Hodgkins lymphoma, adenoma, squamous cell carcinoma, laryngeal carcinoma, gallbladder and bile duct cancers, cancers of the retina such as retinoblastoma, cancers of the esophagus, gastric cancers, multiple myeloma, ovarian cancer, uterine cancer, thyroid cancer, testicular cancer, endometrial cancer, melanoma, colorectal cancer, lung cancer, bladder cancer, prostate cancer, lung cancer (including non-small cell lung carcinoma), pancreatic cancer, sarcomas, Wilms' tumor, cervical cancer, head and neck cancer, skin cancers, nasopharyngeal carcinoma, liposarcoma, epithelial carcinoma, renal cell carcinoma, gallbladder adeno carcinoma, parotid adenocarcinoma, endometrial sarcoma, multidrug resistant cancers; and proliferative diseases and conditions, such as neovascularization associated with tumor angiogenesis, macular degeneration (e.g., wet/dry AMD), corneal neovascularization, diabetic retinopathy, neovascular glaucoma, myopic degeneration and other proliferative diseases and conditions such as restenosis and polycystic kidney disease, and any other cancer or

proliferative disease, condition, trait, genotype or phenotype that can respond to the modulation of disease related gene expression (e.g., XIAP) in a cell or tissue, alone or in combination with other therapies.

By "ocular disease" as used herein is meant, any disease, condition, trait, genotype or phenotype of the eye and related structures, such as Cystoid Macular Edema, Asteroid Hyalosis, Pathological Myopia and Posterior Staphyloma, Toxocariasis (Ocular Larva Migrans), Retinal Vein Occlusion, Posterior Vitreous Detachment, Tractional Retinal Tears, Epiretinal Membrane, Diabetic Retinopathy, Lattice Degeneration, Retinal Vein Occlusion, Retinal Artery Occlusion, Macular Degeneration (e.g., age related macular degeneration such as wet AMD or dry AMD), Toxoplasmosis, Choroidal Melanoma, Acquired Retinoschisis, Hollenhorst Plaque, Idiopathic Central Serous Chorioretinopathy, Macular Hole, Presumed Ocular Histoplasmosis Syndrome, Retinal Macroaneursym, Retinitis Pigmentosa, Retinal Detachment, Hypertensive Retinopathy, Retinal Pigment Epithelium (RPE) Detachment, Papillophlebitis, Ocular Ischemic Syndrome, Coats' Disease, Leber's Miliary Aneurysm, Conjunctival Neoplasms, Allergic Conjunctivitis, Vernal Conjunctivitis, Acute Bacterial Conjunctivitis, Allergic Conjunctivitis &Vernal Keratoconjunctivitis, Viral Conjunctivitis, Bacterial Conjunctivitis, Chlamydial & Gonococcal Conjunctivitis, Conjunctival Laceration, Episcleritis, Scleritis, Pingueculitis, Pterygium, Superior Limbic Keratoconjunctivitis (SLK of Theodore), Toxic Conjunctivitis, Conjunctivitis with Pseudomembrane, Giant Papillary Conjunctivitis, Terrien's Marginal Degeneration, Acanthamoeba Keratitis, Fungal Keratitis, Filamentary Keratitis, Bacterial Keratitis, Keratitis Sicca/Dry Eye Syndrome, Bacterial Keratitis, Herpes Simplex Keratitis, Sterile Corneal Infiltrates, Phlyctenulosis, Corneal Abrasion & Recurrent Corneal Erosion, Corneal Foreign Body, Chemical Burs, Epithelial Basement Membrane Dystrophy (EBMD), Thygeson's Superficial Punctate Keratopathy, Corneal Laceration, Salzmann's Nodular Degeneration, Fuchs' Endothelial Dystrophy, Crystalline Lens Subluxation, Ciliary-Block Glaucoma, Primary Open-Angle Glaucoma, Pigment Dispersion Syndrome and Pigmentary Glaucoma, Pseudoexfoliation Syndrom and Pseudoexfoliative .Glaucoma, Anterior Uveitis, Primary Open Angle Glaucoma, Uveitic Glaucoma & Glaucomatocyclitic Crisis, Pigment Dispersion Syndrome & Pigmentary Glaucoma, Acute Angle Closure Glaucoma, Anterior Uveitis, Hyphema, Angle Recession

Glaucoma, Lens Induced Glaucoma, Pseudoexfoliation Syndrome and Pseudoexfoliative Glaucoma, Axenfeld-Rieger Syndrome, Neovascular Glaucoma, Pars Planitis, Choroidal Rupture, Duane's Retraction Syndrome, Toxic/Nutritional Optic Neuropathy, Aberrant Regeneration of Cranial Nerve III, Intracranial Mass Lesions, Carotid-Cavernous Sinus Fistula, Anterior Ischemic Optic Neuropathy, Optic Disc Edema & Papilledema, Cranial Nerve III Palsy, Cranial Nerve IV Palsy, Cranial Nerve VI Palsy, Cranial Nerve VII (Facial Nerve) Palsy, Horner's Syndrome, Internuclear Ophthalmoplegia, Optic Nerve Head Hypoplasia, Optic Pit, Tonic Pupil, Optic Nerve Head Drusen, Demyelinating Optic Neuropathy (Optic Neuritis, Retrobulbar Optic Neuritis), Amaurosis Fugax and Transient Ischemic Attack, Pseudotumor Cerebri, Pituitary Adenoma, Molluscum Contagiosum, Canaliculitis, Verruca and Papilloma, Pediculosis and Pthiriasis, Blepharitis, Hordeolum, Preseptal Cellulitis, Chalazion, Basal Cell Carcinoma, Herpes Zoster Ophthalmicus, Pediculosis & Phthiriasis, Blow-out Fracture, Chronic Epiphora, Dacryocystitis, Herpes Simplex Blepharitis, Orbital Cellulitis, Senile Entropion, and Squamous Cell Carcinoma.

In one embodiment, the siNA molecules of the invention are used to treat or prevent cancer or other proliferative disorders in a subject or organism.

In one embodiment, the siNA molecules of the invention are used to treat or prevent ocular disease in a subject or organism.

In one embodiment of the present invention, each sequence of a siNA molecule of the invention is independently about 15 to about 30 nucleotides in length, in specific embodiments about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. In another embodiment, the siNA duplexes of the invention independently comprise about 15 to about 30 base pairs (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30). In another embodiment, one or more strands of the siNA molecule of the invention independently comprises about 15 to about 30 nucleotides (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) that are complementary to a target nucleic acid molecule. In yet another embodiment, siNA molecules of the invention comprising hairpin or circular structures are about 35 to about 55 (*e.g.*, about 35, 40, 45, 50 or 55) nucleotides in length, or about 38 to about 44 (*e.g.*, about 38, 39, 40, 41, 42, 43, or 44) nucleotides in length and comprising about 15

to about 25 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs. Exemplary siNA molecules of the invention are shown in **Table II**. Exemplary synthetic siNA molecules of the invention are shown in **Table III** and/or **Figures 4-5**.

As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human. The cell can be present in an organism, e.g., birds, plants and mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell). The cell can be of somatic or germ line origin, totipotent or pluripotent, dividing or non-dividing. The cell can also be derived from or can comprise a gamete or embryo, a stem cell, or a fully differentiated cell.

The siNA molecules of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through direct dermal application, transdermal application, or injection, with or without their incorporation in biopolymers. In particular embodiments, the nucleic acid molecules of the invention comprise sequences shown in **Tables II-III** and/or **Figures 4-5**. Examples of such nucleic acid molecules consist essentially of sequences defined in these tables and figures. Furthermore, the chemically modified constructs described in **Table IV** can be applied to any siNA sequence of the invention.

In another aspect, the invention provides mammalian cells containing one or more siNA molecules of this invention. The one or more siNA molecules can independently be targeted to the same or different sites.

By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a β-D-ribofuranose moiety. The terms include double-stranded RNA, single-stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siNA or internally, for example at one or more nucleotides of the RNA.

Nucleotides in the RNA molecules of the instant invention can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

By "subject" is meant an organism, which is a donor or recipient of explanted cells or the cells themselves. "Subject" also refers to an organism to which the nucleic acid molecules of the invention can be administered. A subject can be a mammal or mammalian cells, including a human or human cells.

The term "phosphorothioate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise a sulfur atom. Hence, the term phosphorothioate refers to both phosphorothioate and phosphorodithioate internucleotide linkages.

The term "phosphonoacetate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise an acetyl or protected acetyl group.

The term "thiophosphonoacetate" as used herein refers to an internucleotide linkage having Formula I, wherein Z comprises an acetyl or protected acetyl group and W comprises a sulfur atom or alternately W comprises an acetyl or protected acetyl group and Z comprises a sulfur atom.

The term "universal base" as used herein refers to nucleotide base analogs that form base pairs with each of the natural DNA/RNA bases with little discrimination between them. Non-limiting examples of universal bases include C-phenyl, C-naphthyl and other aromatic derivatives, inosine, azole carboxamides, and nitroazole derivatives such as 3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole as known in the art (see for example Loakes, 2001, *Nucleic Acids Research,* 29, 2437-2447).

The term "acyclic nucleotide" as used herein refers to any nucleotide having an acyclic ribose sugar, for example where any of the ribose carbons (C1, C2, C3, C4, or C5), are independently or in combination absent from the nucleotide.

The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to for preventing or treating cancer or other proliferative disorder in a subject or organism.

For example, the siNA molecules can be administered to a subject or can be administered to other appropriate cells evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

In a further embodiment, the siNA molecules can be used in combination with other known treatments to prevent or treat cancer or other proliferative disorder in a subject or organism. For example, the described molecules could be used in combination with one or more known compounds, treatments, or procedures to prevent or treat cancer or other proliferative disorder in a subject or organism as are known in the art.

In one embodiment, the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention, in a manner which allows expression of the siNA molecule. For example, the vector can contain sequence(s) encoding both strands of a siNA molecule comprising a duplex. The vector can also contain sequence(s) encoding a single nucleic acid molecule that is self-complementary and thus forms a siNA molecule. Non-limiting examples of such expression vectors are described in Paul *et al.*, 2002, *Nature Biotechnology*, 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology*, 19, 497; Lee *et al.*, 2002, *Nature Biotechnology*, 19, 500; and Novina *et al.*, 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725.

In another embodiment, the invention features a mammalian cell, for example, a human cell, including an expression vector of the invention.

In yet another embodiment, the expression vector of the invention comprises a sequence for a siNA molecule having complementarity to a RNA molecule referred to by a Genbank Accession numbers, for example Genbank Accession Nos. shown in **Table I**.

In one embodiment, an expression vector of the invention comprises a nucleic acid sequence encoding two or more siNA molecules, which can be the same or different.

In another aspect of the invention, siNA molecules that interact with target RNA molecules and down-regulate gene encoding target RNA molecules (for example target RNA molecules referred to by Genbank Accession numbers herein) are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecules bind and down-regulate gene function or expression via RNA interference (RNAi). Delivery of siNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a non-limiting example of a scheme for the synthesis of siNA molecules. The complementary siNA sequence strands, strand 1 and strand 2, are synthesized in tandem and are connected by a cleavable linkage, such as a nucleotide succinate or abasic succinate, which can be the same or different from the cleavable linker used for solid phase synthesis on a solid support. The synthesis can be either solid phase or solution phase, in the example shown, the synthesis is a solid phase synthesis. The synthesis is performed such that a protecting group, such as a dimethoxytrityl group, remains intact on the terminal nucleotide of the tandem oligonucleotide. Upon cleavage and deprotection of the oligonucleotide, the two siNA strands spontaneously hybridize to form a siNA duplex, which allows the purification of the duplex by utilizing the properties of the terminal protecting group, for example by applying a trityl on

purification method wherein only duplexes/oligonucleotides with the terminal protecting group are isolated.

Figure 2 shows a MALDI-TOF mass spectrum of a purified siNA duplex synthesized by a method of the invention. The two peaks shown correspond to the predicted mass of the separate siNA sequence strands. This result demonstrates that the siNA duplex generated from tandem synthesis can be purified as a single entity using a simple trityl-on purification methodology.

Figure 3 shows a non-limiting proposed mechanistic representation of target RNA degradation involved in RNAi. Double-stranded RNA (dsRNA), which is generated by RNA-dependent RNA polymerase (RdRP) from foreign single-stranded RNA, for example viral, transposon, or other exogenous RNA, activates the DICER enzyme that in turn generates siNA duplexes. Alternately, synthetic or expressed siNA can be introduced directly into a cell by appropriate means. An active siNA complex forms which recognizes a target RNA, resulting in degradation of the target RNA by the RISC endonuclease complex or in the synthesis of additional RNA by RNA-dependent RNA polymerase (RdRP), which can activate DICER and result in additional siNA molecules, thereby amplifying the RNAi response.

Figure 4A-F shows non-limiting examples of chemically-modified siNA constructs of the present invention. In the figure, N stands for any nucleotide (adenosine, guanosine, cytosine, uridine, or optionally thymidine, for example thymidine can be substituted in the overhanging regions designated by parenthesis (N N). Various modifications are shown for the sense and antisense strands of the siNA constructs.

Figure 4A: The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all nucleotides present are ribonucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all nucleotides present are ribonucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide

linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

Figure 4B: The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the sense and antisense strand.

Figure 4C: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

Figure 4D: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

Figure 4E: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

Figure 4F: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified

nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-deoxy nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand. The antisense strand of constructs A-F comprise sequence complementary to any target nucleic acid sequence of the invention. Furthermore, when a glyceryl moiety (L) is present at the 3'-end of the antisense strand for any construct shown in **Figure 4 A-F**, the modified internucleotide linkage is optional.

Figure 5A-F shows non-limiting examples of specific chemically-modified siNA sequences of the invention. **A-F** applies the chemical modifications described in **Figure 4A-F** to a XIAP siNA sequence. Such chemical modifications can be applied to any XIAP sequence and/or XIAP polymorphism sequence.

Figure 6 shows non-limiting examples of different siNA constructs of the invention. The examples shown (constructs 1, 2, and 3) have 19 representative base pairs; however, different embodiments of the invention include any number of base pairs described herein. Bracketed regions represent nucleotide overhangs, for example, comprising about 1, 2, 3, or 4 nucleotides in length, preferably about 2 nucleotides. Constructs 1 and 2 can be used independently for RNAi activity. Construct 2 can comprise a polynucleotide or non-nucleotide linker, which can optionally be designed as a biodegradable linker. In one embodiment, the loop structure shown in construct 2 can comprise a biodegradable linker that results in the formation of construct 1 *in vivo* and/or *in vitro*. In another example, construct 3 can be used to generate construct 2 under the same principle wherein a linker is used to generate the active siNA construct 2 *in vivo*

and/or *in vitro*, which can optionally utilize another biodegradable linker to generate the active siNA construct 1 *in vivo* and/or *in vitro*. As such, the stability and/or activity of the siNA constructs can be modulated based on the design of the siNA construct for use *in vivo* or *in vitro* and/or *in vitro*.

Figure 7A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate siNA hairpin constructs.

Figure 7A: A DNA oligomer is synthesized with a 5'-restriction site (R1) sequence followed by a region having sequence identical (sense region of siNA) to a predetermined XIAP target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, which is followed by a loop sequence of defined sequence (X), comprising, for example, about 3 to about 10 nucleotides.

Figure 7B: The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence that will result in a siNA transcript having specificity for a XIAP target sequence and having self-complementary sense and antisense regions.

Figure 7C: The construct is heated (for example to about 95°C) to linearize the sequence, thus allowing extension of a complementary second DNA strand using a primer to the 3'-restriction sequence of the first strand. The double-stranded DNA is then inserted into an appropriate vector for expression in cells. The construct can be designed such that a 3'-terminal nucleotide overhang results from the transcription, for example, by engineering restriction sites and/or utilizing a poly-U termination region as described in Paul *et al.*, 2002, *Nature Biotechnology*, 29, 505-508.

Figure 8A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate double-stranded siNA constructs.

Figure 8A: A DNA oligomer is synthesized with a 5'-restriction (R1) site sequence followed by a region having sequence identical (sense region of siNA) to a predetermined XIAP target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, and which is followed by a 3'-restriction site (R2) which is adjacent to a loop sequence of defined sequence (X).

Figure 8B: The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence.

Figure 8C: The construct is processed by restriction enzymes specific to R1 and R2 to generate a double-stranded DNA which is then inserted into an appropriate vector for expression in cells. The transcription cassette is designed such that a U6 promoter region flanks each side of the dsDNA which generates the separate sense and antisense strands of the siNA. Poly T termination sequences can be added to the constructs to generate U overhangs in the resulting transcript.

Figure 9A-E is a diagrammatic representation of a method used to determine target sites for siNA mediated RNAi within a particular target nucleic acid sequence, such as messenger RNA.

Figure 9A: A pool of siNA oligonucleotides are synthesized wherein the antisense region of the siNA constructs has complementarity to target sites across the target nucleic acid sequence, and wherein the sense region comprises sequence complementary to the antisense region of the siNA.

Figure 9B&C: (**Figure 9B**) The sequences are pooled and are inserted into vectors such that (**Figure 9C**) transfection of a vector into cells results in the expression of the siNA.

Figure 9D: Cells are sorted based on phenotypic change that is associated with modulation of the target nucleic acid sequence.

Figure 9E: The siNA is isolated from the sorted cells and is sequenced to identify efficacious target sites within the target nucleic acid sequence.

Figure 10 shows non-limiting examples of different stabilization chemistries (1-10) that can be used, for example, to stabilize the 3'-end of siNA sequences of the invention, including **(1)** [3-3']-inverted deoxyribose; **(2)** deoxyribonucleotide; **(3)** [5'-3']-3'-deoxyribonucleotide; **(4)** [5'-3']-ribonucleotide; **(5)** [5'-3']-3'-O-methyl ribonucleotide; **(6)** 3'-glyceryl; **(7)** [3'-5']-3'-deoxyribonucleotide; **(8)** [3'-3']-deoxyribonucleotide; **(9)** [5'-2']-deoxyribonucleotide; and **(10)** [5-3']-dideoxyribonucleotide. In addition to modified and unmodified backbone chemistries indicated in the figure, these chemistries can be

combined with different backbone modifications as described herein, for example, backbone modifications having Formula I. In addition, the 2'-deoxy nucleotide shown 5' to the terminal modifications shown can be another modified or unmodified nucleotide or non-nucleotide described herein, for example modifications having any of Formulae I-VII or any combination thereof.

Figure 11 shows a non-limiting example of a strategy used to identify chemically modified siNA constructs of the invention that are nuclease resistance while preserving the ability to mediate RNAi activity. Chemical modifications are introduced into the siNA construct based on educated design parameters (e.g. introducing 2'-mofications, base modifications, backbone modifications, terminal cap modifications etc). The modified construct in tested in an appropriate system (e.g. human serum for nuclease resistance, shown, or an animal model for PK/delivery parameters). In parallel, the siNA construct is tested for RNAi activity, for example in a cell culture system such as a luciferase reporter assay). Lead siNA constructs are then identified which possess a particular characteristic while maintaining RNAi activity, and can be further modified and assayed once again. This same approach can be used to identify siNA-conjugate molecules with improved pharmacokinetic profiles, delivery, and RNAi activity.

Figure 12 shows non-limiting examples of phosphorylated siNA molecules of the invention, including linear and duplex constructs and asymmetric derivatives thereof.

Figure 13 shows non-limiting examples of chemically modified terminal phosphate groups of the invention.

Figure 14A shows a non-limiting example of methodology used to design self complementary DFO constructs utilizing palidrome and/or repeat nucleic acid sequences that are identified in a target nucleic acid sequence. (i) A palindrome or repeat sequence is identified in a nucleic acid target sequence. (ii) A sequence is designed that is complementary to the target nucleic acid sequence and the palindrome sequence. (iii) An inverse repeat sequence of the non-palindrome/repeat portion of the complementary sequence is appended to the 3'-end of the complementary sequence to generate a self complementary DFO molecule comprising sequence complementary to the nucleic acid target. (iv) The DFO molecule can self-assemble to form a double stranded oligonucleotide. **Figure 14B** shows a non-limiting representative example of a duplex

forming oligonucleotide sequence. **Figure 14C** shows a non-limiting example of the self assembly schematic of a representative duplex forming oligonucleotide sequence. **Figure 14D** shows a non-limiting example of the self assembly schematic of a representative duplex forming oligonucleotide sequence followed by interaction with a target nucleic acid sequence resulting in modulation of gene expression.

Figure 15 shows a non-limiting example of the design of self complementary DFO constructs utilizing palidrome and/or repeat nucleic acid sequences that are incorporated into the DFO constructs that have sequence complementary to any target nucleic acid sequence of interest. Incorporation of these palindrome/repeat sequences allow the design of DFO constructs that form duplexes in which each strand is capable of mediating modulation of target gene expression, for example by RNAi. First, the target sequence is identified. A complementary sequence is then generated in which nucleotide or non-nucleotide modifications (shown as X or Y) are introduced into the complementary sequence that generate an artificial palindrome (shown as XYXYXY in the Figure). An inverse repeat of the non-palindrome/repeat complementary sequence is appended to the 3'-end of the complementary sequence to generate a self complementary DFO comprising sequence complementary to the nucleic acid target. The DFO can self-assemble to form a double stranded oligonucleotide.

Figure 16 shows non-limiting examples of multifunctional siNA molecules of the invention comprising two separate polynucleotide sequences that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences. **Figure 16A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 3'-ends of each polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 16B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid

sequence (complementary region 2), wherein the first and second complementary regions are situated at the 5'-ends of each polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences.

Figure 17 shows non-limiting examples of multifunctional siNA molecules of the invention comprising a single polynucleotide sequence comprising distinct regions that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences. **Figure 17A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the second complementary region is situated at the 3'-end of the polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 17B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first complementary region is situated at the 5'-end of the polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. In one embodiment, these multifunctional siNA constructs are processed in vivo or in vitro to generate multifunctional siNA constructs as shown in **Figure 16.**

Figure 18 shows non-limiting examples of multifunctional siNA molecules of the invention comprising two separate polynucleotide sequences that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences and wherein the multifunctional siNA construct further comprises a self complementary, palindrome, or repeat region, thus enabling shorter bifuctional siNA constructs that can mediate RNA interference against differing target nucleic acid sequences. **Figure 18A** shows a non-

limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 3'-ends of each polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 18B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 5'-ends of each polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences.

Figure 19 shows non-limiting examples of multifunctional siNA molecules of the invention comprising a single polynucleotide sequence comprising distinct regions that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences and wherein the multifunctional siNA construct further comprises a self complementary, palindrome, or repeat region, thus enabling shorter bifuctional siNA constructs that can mediate RNA interference against differing target nucleic acid sequences. **Figure 19A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the second complementary region is situated at the 3'-end of the polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of

each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 19B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first complementary region is situated at the 5'-end of the polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. In one embodiment, these multifunctional siNA constructs are processed in vivo or in vitro to generate multifunctional siNA constructs as shown in **Figure 18**.

Figure 20 shows a non-limiting example of how multifunctional siNA molecules of the invention can target two separate target nucleic acid molecules, such as separate RNA molecules encoding differing proteins, for example, a cytokine and its corresponding receptor, differing viral strains, a virus and a cellular protein involved in viral infection or replication, or differing proteins involved in a common or divergent biologic pathway that is implicated in the maintenance of progression of disease. Each strand of the multifunctional siNA construct comprises a region having complementarity to separate target nucleic acid molecules. The multifunctional siNA molecule is designed such that each strand of the siNA can be utilized by the RISC complex to initiate RNA interference mediated cleavage of its corresponding target. These design parameters can include destabilization of each end of the siNA construct (see for example Schwarz *et al.*, 2003, *Cell*, 115, 199-208). Such destabilization can be accomplished for example by using guanosine-cytidine base pairs, alternate base pairs (e.g., wobbles), or destabilizing chemically modified nucleotides at terminal nucleotide positions as is known in the art.

Figure 21 shows a non-limiting example of how multifunctional siNA molecules of the invention can target two separate target nucleic acid sequences within the same target nucleic acid molecule, such as alternate coding regions of a RNA, coding and non-

coding regions of a RNA, or alternate splice variant regions of a RNA. Each strand of the multifunctional siNA construct comprises a region having complementarity to the separate regions of the target nucleic acid molecule. The multifunctional siNA molecule is designed such that each strand of the siNA can be utilized by the RISC complex to initiate RNA interference mediated cleavage of its corresponding target region. These design parameters can include destabilization of each end of the siNA construct (see for example Schwarz *et al.*, 2003, *Cell*, 115, 199-208). Such destabilization can be accomplished for example by using guanosine-cytidine base pairs, alternate base pairs (e.g., wobbles), or destabilizing chemically modified nucleotides at terminal nucleotide positions as is known in the art.

Figure 22 shows a non-limiting example of reduction of XIAP mRNA in A549 cells mediated by chemically modified siNAs that target XIAP mRNA. A549 cells were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. Active siNA constructs comprising various stabilization chemistries (see Tables III and IV) were compared to untreated cells, matched chemistry irrelevant siNA control constructs (IC1, IC2), and cells transfected with lipid alone (transfection control). As shown in the figure, the siNA constructs significantly reduce XIAP RNA expression.

DETAILED DESCRIPTION OF THE INVENTION

Mechanism of Action of Nucleic Acid Molecules of the Invention

The discussion that follows discusses the proposed mechanism of RNA interference mediated by short interfering RNA as is presently known, and is not meant to be limiting and is not an admission of prior art. Applicant demonstrates herein that chemically-modified short interfering nucleic acids possess similar or improved capacity to mediate RNAi as do siRNA molecules and are expected to possess improved stability and activity *in vivo*; therefore, this discussion is not meant to be limiting only to siRNA and can be applied to siNA as a whole. By "improved capacity to mediate RNAi" or "improved RNAi activity" is meant to include RNAi activity measured *in vitro* and/or *in vivo* where the RNAi activity is a reflection of both the ability of the siNA to mediate RNAi and the stability of the siNAs of the invention. In this invention, the product of these activities can be increased *in vitro* and/or *in vivo* compared to an all RNA siRNA or a siNA containing a plurality of ribonucleotides. In some cases, the activity or stability

of the siNA molecule can be decreased (i.e., less than ten-fold), but the overall activity of the siNA molecule is enhanced *in vitro* and/or *in vivo*.

RNA interference refers to the process of sequence specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire *et al.*, 1998, *Nature*, 391, 806). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes which is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response though a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2', 5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as Dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Berstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from Dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes. Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence homologous to the siRNA. Cleavage of the target RNA takes place in the middle of the region complementary to the guide sequence of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). In addition, RNA interference can also involve small RNA (e.g., micro-RNA or miRNA) mediated gene silencing,

presumably though cellular mechanisms that regulate chromatin structure and thereby prevent transcription of target gene sequences (see for example Allshire, 2002, *Science,* 297, 1818-1819; Volpe *et al.,* 2002, *Science,* 297, 1833-1837; Jenuwein, 2002, *Science,* 297, 2215-2218; and Hall *et al.,* 2002, *Science,* 297, 2232-2237). As such, siNA molecules of the invention can be used to mediate gene silencing via interaction with RNA transcripts or alternately by interaction with particular gene sequences, wherein such interaction results in gene silencing either at the transcriptional level or post-transcriptional level.

RNAi has been studied in a variety of systems. Fire *et al.,* 1998, *Nature,* 391, 806, were the first to observe RNAi in *C. elegans*. Wianny and Goetz, 1999, *Nature Cell Biol.,* 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond *et al.,* 2000, *Nature,* 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.,* 2001, *Nature,* 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in Drosophila embryonic lysates has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21 nucleotide siRNA duplexes are most active when containing two 2-nucleotide 3'-terminal nucleotide overhangs. Furthermore, substitution of one or both siRNA strands with 2'-deoxy or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of 3'-terminal siRNA nucleotides with deoxy nucleotides was shown to be tolerated. Mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end (Elbashir *et al.,* 2001, *EMBO J.,* 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.,* 2001, *Cell,* 107, 309); however, siRNA molecules lacking a 5'-phosphate are active when introduced exogenously, suggesting that 5'-phosphorylation of siRNA constructs may occur *in vivo*.

Synthesis of Nucleic Acid Molecules

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs ("small" refers to nucleic acid motifs no more than 100 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; *e.g.*, individual siNA oligonucleotide sequences or siNA sequences synthesized in tandem) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of protein and/or RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

Oligonucleotides (*e.g.*, certain modified oligonucleotides or portions of oligonucleotides lacking ribonucleotides) are synthesized using protocols known in the art, for example as described in Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19, Thompson *et al.*, International PCT Publication No. WO 99/54459, Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, Brennan *et al.*, 1998, *Biotechnol Bioeng.*, 61, 33-45, and Brennan, U.S. Pat. No. 6,001,311. All of these references are incorporated herein by reference. The synthesis of oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 second coupling step for 2'-deoxy nucleotides or 2'-deoxy-2'-fluoro nucleotides. **Table V** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μL of 0.11 M = 6.6 μmol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60 μL of 0.25 M = 15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 22-fold excess (40 μL of 0.11 M = 4.4 μmol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40 μL of 0.25 M = 10 μmol) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are

typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PerSeptive Biosystems, Inc.). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

Deprotection of the DNA-based oligonucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aqueous methylamine (1 mL) at 65 °C for 10 minutes. After cooling to –20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder.

The method of synthesis used for RNA including certain siNA molecules of the invention follows the procedure as described in Usman *et al.*, 1987, *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.*, 1990, *Nucleic Acids Res.*, 18, 5433; and Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684 Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 µmol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. **Table V** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 µmol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 µL of 0.11 M = 6.6 µmol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60 µL of 0.25 M = 15 µmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-

bound 5'-hydroxyl. A 66-fold excess (120 μL of 0.11 M = 13.2 μmol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120 μL of 0.25 M = 30 μmol) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PerSeptive Biosystems, Inc.). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide0.05 M in acetonitrile) is used.

Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to –20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300 μL of a solution of 1.5 mL N-methylpyrrolidinone, 750 μL TEA and 1 mL TEA•3HF to provide a 1.4 M HF concentration) and heated to 65 °C. After 1.5 h, the oligomer is quenched with 1.5 M NH_4HCO_3.

Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65 °C for 15 minutes. The vial is brought to room temperature TEA•3HF (0.1 mL) is added and the vial is heated at 65 °C for 15 minutes. The sample is cooled at –20 °C and then quenched with 1.5 M NH_4HCO_3.

For purification of the trityl-on oligomers, the quenched NH_4HCO_3 solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA for 13 minutes. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

The average stepwise coupling yields are typically >98% (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described above including but not limited to 96-well format.

Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example, by ligation (Moore *et al.*, 1992, *Science* 256, 9923; Draper *et al.*, International PCT publication No. WO 93/23569; Shabarova *et al.*, 1991, *Nucleic Acids Research* 19, 4247; Bellon *et al.*, 1997, *Nucleosides & Nucleotides*, 16, 951; Bellon *et al.*, 1997, *Bioconjugate Chem.* 8, 204), or by hybridization following synthesis and/or deprotection.

The siNA molecules of the invention can also be synthesized via a tandem synthesis methodology as described in Example 1 herein, wherein both siNA strands are synthesized as a single contiguous oligonucleotide fragment or strand separated by a cleavable linker which is subsequently cleaved to provide separate siNA fragments or strands that hybridize and permit purification of the siNA duplex. The linker can be a polynucleotide linker or a non-nucleotide linker. The tandem synthesis of siNA as described herein can be readily adapted to both multiwell/multiplate synthesis platforms such as 96 well or similarly larger multi-well platforms. The tandem synthesis of siNA as described herein can also be readily adapted to large scale synthesis platforms employing batch reactors, synthesis columns and the like.

A siNA molecule can also be assembled from two distinct nucleic acid strands or fragments wherein one fragment includes the sense region and the second fragment includes the antisense region of the RNA molecule.

The nucleic acid molecules of the present invention can be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-fluoro, 2'-*O*-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163). siNA constructs can be purified by gel electrophoresis using general methods or can be purified by high pressure liquid chromatography (HPLC; see Wincott *et al., supra*, the totality of which is hereby incorporated herein by reference) and re-suspended in water.

In another aspect of the invention, siNA molecules of the invention are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules.

Optimizing Activity of the nucleic acid molecule of the invention.

Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) can prevent their degradation by serum ribonucleases, which can increase their potency (see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 *Nature* 344, 565; Pieken *et al.*, 1991, *Science* 253, 314; Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162; Sproat, U.S. Pat. No. 5,334,711; Gold *et al.*, U.S. Pat. No. 6,300,074; and Burgin *et al., supra;* all of which are incorporated by reference herein). All of the above references describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules described herein. Modifications that enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are

modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-fluoro, 2'-*O*-methyl, 2'-O-allyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, *TIBS.* 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin *et al.*, 1996, *Biochemistry*, 35, 14090). Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein *et al.*, *International Publication* PCT No. WO 92/07065; Perrault *et al. Nature*, 1990, *344*, 565-568; Pieken *et al. Science*, 1991, *253*, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.*, 1992, *17*, 334-339; Usman *et al. International Publication* PCT No. WO 93/15187; Sproat, *U.S. Pat.* No. 5,334,711 and Beigelman *et al.*, 1995, *J. Biol. Chem.*, 270, 25702; Beigelman *et al.*, International PCT publication No. WO 97/26270; Beigelman *et al.*, U.S. Pat. No. 5,716,824; Usman *et al.*, U.S. Pat. No. 5,627,053; Woolf *et al.*, International PCT Publication No. WO 98/13526; Thompson *et al.*, USSN 60/082,404 which was filed on April 20, 1998; Karpeisky *et al.*, 1998, *Tetrahedron Lett.*, 39, 1131; Earnshaw and Gait, 1998, *Biopolymers (Nucleic Acid Sciences)*, 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.*, 67, 99-134; and Burlina *et al.*, 1997, *Bioorg. Med. Chem.*, 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into nucleic acid molecules without modulating catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify the siNA nucleic acid molecules of the instant invention so long as the ability of siNA to promote RNAi is cells is not significantly inhibited.

While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorodithioate, and/or 5'-methylphosphonate linkages improves stability, excessive modifications can cause some toxicity or decreased activity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages should be minimized. The reduction in the concentration of these linkages should lower toxicity, resulting in increased efficacy and higher specificity of these molecules.

Short interfering nucleic acid (siNA) molecules having chemical modifications that maintain or enhance activity are provided. Such a nucleic acid is also generally more

resistant to nucleases than an unmodified nucleic acid. Accordingly, the *in vitro* and/or *in vivo* activity should not be significantly lowered. In cases in which modulation is the goal, therapeutic nucleic acid molecules delivered exogenously should optimally be stable within cells until translation of the target RNA has been modulated long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Improvements in the chemical synthesis of RNA and DNA (Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677; Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19 (incorporated by reference herein)) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability, as described above.

In one embodiment, nucleic acid molecules of the invention include one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) G-clamp nucleotides. A G-clamp nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine within a duplex, see for example Lin and Matteucci, 1998, *J. Am. Chem. Soc.*, 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in nucleic acid molecules of the invention results in both enhanced affinity and specificity to nucleic acid targets, complementary sequences, or template strands. In another embodiment, nucleic acid molecules of the invention include one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) LNA "locked nucleic acid" nucleotides such as a 2', 4'-C methylene bicyclo nucleotide (see for example Wengel *et al.*, International PCT Publication No. WO 00/66604 and WO 99/14226).

In another embodiment, the invention features conjugates and/or complexes of siNA molecules of the invention. Such conjugates and/or complexes can be used to facilitate delivery of siNA molecules into a biological system, such as a cell. The conjugates and complexes provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes, altering the pharmacokinetics, and/or modulating the localization of nucleic acid molecules of the invention. The present invention encompasses the design and synthesis of novel conjugates and complexes for the delivery of molecules, including, but not limited to,

small molecules, lipids, cholesterol, phospholipids, nucleosides, nucleotides, nucleic acids, antibodies, toxins, negatively charged polymers and other polymers, for example proteins, peptides, hormones, carbohydrates, polyethylene glycols, or polyamines, across cellular membranes. In general, the transporters described are designed to be used either individually or as part of a multi-component system, with or without degradable linkers. These compounds are expected to improve delivery and/or localization of nucleic acid molecules of the invention into a number of cell types originating from different tissues, in the presence or absence of serum (see Sullenger and Cech, U.S. Pat. No. 5,854,038). Conjugates of the molecules described herein can be attached to biologically active molecules via linkers that are biodegradable, such as biodegradable nucleic acid linker molecules.

The term "biodegradable linker" as used herein, refers to a nucleic acid or non-nucleic acid linker molecule that is designed as a biodegradable linker to connect one molecule to another molecule, for example, a biologically active molecule to a siNA molecule of the invention or the sense and antisense strands of a siNA molecule of the invention. The biodegradable linker is designed such that its stability can be modulated for a particular purpose, such as delivery to a particular tissue or cell type. The stability of a nucleic acid-based biodegradable linker molecule can be modulated by using various chemistries, for example combinations of ribonucleotides, deoxyribonucleotides, and chemically-modified nucleotides, such as 2'-O-methyl, 2'-fluoro, 2'-amino, 2'-O-amino, 2'-C-allyl, 2'-O-allyl, and other 2'-modified or base modified nucleotides. The biodegradable nucleic acid linker molecule can be a dimer, trimer, tetramer or longer nucleic acid molecule, for example, an oligonucleotide of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length, or can comprise a single nucleotide with a phosphorus-based linkage, for example, a phosphoramidate or phosphodiester linkage. The biodegradable nucleic acid linker molecule can also comprise nucleic acid backbone, nucleic acid sugar, or nucleic acid base modifications.

The term "biodegradable" as used herein, refers to degradation in a biological system, for example, enzymatic degradation or chemical degradation.

The term "biologically active molecule" as used herein refers to compounds or molecules that are capable of eliciting or modifying a biological response in a system.

Non-limiting examples of biologically active siNA molecules either alone or in combination with other molecules contemplated by the instant invention include therapeutically active molecules such as antibodies, cholesterol, hormones, antivirals, peptides, proteins, chemotherapeutics, small molecules, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, 2,5-A chimeras, siNA, dsRNA, allozymes, aptamers, decoys and analogs thereof. Biologically active molecules of the invention also include molecules capable of modulating the pharmacokinetics and/or pharmacodynamics of other biologically active molecules, for example, lipids and polymers such as polyamines, polyamides, polyethylene glycol and other polyethers.

The term "phospholipid" as used herein, refers to a hydrophobic molecule comprising at least one phosphorus group. For example, a phospholipid can comprise a phosphorus-containing group and saturated or unsaturated alkyl group, optionally substituted with OH, COOH, oxo, amine, or substituted or unsubstituted aryl groups.

Therapeutic nucleic acid molecules (*e.g.,* siNA molecules) delivered exogenously optimally are stable within cells until reverse transcription of the RNA has been modulated long enough to reduce the levels of the RNA transcript. The nucleic acid molecules are resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of nucleic acid molecules described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

In yet another embodiment, siNA molecules having chemical modifications that maintain or enhance enzymatic activity of proteins involved in RNAi are provided. Such nucleic acids are also generally more resistant to nucleases than unmodified nucleic acids. Thus, *in vitro* and/or *in vivo* the activity should not be significantly lowered.

Use of the nucleic acid-based molecules of the invention will lead to better treatments by affording the possibility of combination therapies (*e.g.,* multiple siNA molecules targeted to different genes; nucleic acid molecules coupled with known small molecule modulators; or intermittent treatment with combinations of molecules, including different motifs and/or other chemical or biological molecules). The treatment

of subjects with siNA molecules can also include combinations of different types of nucleic acid molecules, such as enzymatic nucleic acid molecules (ribozymes), allozymes, antisense, 2,5-A oligoadenylate, decoys, and aptamers.

In another aspect a siNA molecule of the invention comprises one or more 5' and/or a 3'- cap structure, for example, on only the sense siNA strand, the antisense siNA strand, or both siNA strands.

By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see, for example, Adamic *et al.*, U.S. Pat. No. 5,998,203, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and may help in delivery and/or localization within a cell. The cap may be present at the 5'-terminus (5'-cap) or at the 3'-terminal (3'-cap) or may be present on both termini. In non-limiting examples, the 5'-cap includes, but is not limited to, glyceryl, inverted deoxy abasic residue (moiety); 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide; carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety. Non-limiting examples of cap moieties are shown in Figure 10.

Non-limiting examples of the 3'-cap include, but are not limited to, glyceryl, inverted deoxy abasic residue (moiety), 4', 5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate; 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide

moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein).

By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine and therefore lacks a base at the 1'-position.

An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino, or SH. The term also includes alkenyl groups that are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably, it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2, halogen, $N(CH_3)_2$, amino, or SH. The term "alkyl" also includes alkynyl groups that have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably, it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino or SH.

Such alkyl groups can also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group that has at

least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

By "nucleotide" as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see, for example, Usman and McSwiggen, *supra*; Eckstein *et al.*, International PCT Publication No. WO 92/07065; Usman *et al.*, International PCT Publication No. WO 93/15187; Uhlman & Peyman, *supra*, all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al.*, 1994, *Nucleic Acids Res.* 22, 2183. Some of the non-limiting examples of base modifications that can be introduced into nucleic acid molecules include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (*e.g.*, 5-methylcytidine), 5-alkyluridines (*e.g.*, ribothymidine), 5-halouridine (*e.g.*, 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (*e.g.* 6-methyluridine), propyne, and others (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090; Uhlman & Peyman, *supra*). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents.

In one embodiment, the invention features modified siNA molecules, with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, phosphotriester, morpholino, amidate carbamate, carboxymethyl, acetamidate, polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications, see Hunziker and Leumann, 1995, *Nucleic Acid Analogues: Synthesis and Properties,* in *Modern Synthetic Methods,* VCH, 331-417, and Mesmaeker *et al.,* 1994, *Novel Backbone Replacements for Oligonucleotides,* in *Carbohydrate Modifications in Antisense Research,* ACS, 24-39.

By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, see for example Adamic *et al.,* U.S. Pat. No. 5,998,203.

By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, thymine, or uracil joined to the 1' carbon of β-D-ribo-furanose.

By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate. Non-limiting examples of modified nucleotides are shown by Formulae I-VII and/or other modifications described herein.

In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH_2 or 2'-*O*- NH_2, which can be modified or unmodified. Such modified groups are described, for example, in Eckstein *et al.,* U.S. Pat. No. 5,672,695 and Matulic-Adamic *et al.,* U.S. Pat. No. 6,248,878, which are both incorporated by reference in their entireties.

Various modifications to nucleic acid siNA structure can be made to enhance the utility of these molecules. Such modifications will enhance shelf-life, half-life *in vitro,* stability, and ease of introduction of such oligonucleotides to the target site, *e.g.,* to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

Administration of Nucleic Acid Molecules

A siNA molecule of the invention can be adapted for use to prevent or treat cancer or other proliferative disorders and conditions, ocular disease, or any other trait, disease or condition that is related to or will respond to the levels of XIAP in a cell or tissue, alone or in combination with other therapies. For example, a siNA molecule can comprise a delivery vehicle, including liposomes, for administration to a subject, carriers and diluents and their salts, and/or can be present in pharmaceutically acceptable formulations. Methods for the delivery of nucleic acid molecules are described in Akhtar *et al.*, 1992, *Trends Cell Bio.*, 2, 139; *Delivery Strategies for Antisense Oligonucleotide Therapeutics*, ed. Akhtar, 1995, Maurer *et al.*, 1999, *Mol. Membr. Biol.*, 16, 129-140; Hofland and Huang, 1999, *Handb. Exp. Pharmacol.*, 137, 165-192; and Lee *et al.*, 2000, *ACS Symp. Ser.*, 752, 184-192, all of which are incorporated herein by reference. Beigelman *et al.*, U.S. Pat. No. 6,395,713 and Sullivan *et al.*, PCT WO 94/02595 further describe the general methods for delivery of nucleic acid molecules. These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules can be administered to cells by a variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as biodegradable polymers, hydrogels, cyclodextrins (see for example Gonzalez *et al.*, 1999, *Bioconjugate Chem.*, 10, 1068-1074; Wang *et al.*, International PCT publication Nos. WO 03/47518 and WO 03/46185), poly(lactic-co-glycolic)acid (PLGA) and PLCA microspheres (see for example US Patent 6,447,796 and US Patent Application Publication No. US 2002130430), biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO 00/53722). In another embodiment, the nucleic acid molecules of the invention can also be formulated or complexed with polyethyleneimine and derivatives thereof, such as polyethyleneimine-polyethyleneglycol-N-acetylgalactosamine (PEI-PEG-GAL) or polyethyleneimine-polyethyleneglycol-tri-N-acetylgalactosamine (PEI-PEG-triGAL) derivatives. In one embodiment, the nucleic acid molecules of the invention are formulated as described in United States Patent Application Publication No. 20030077829, incorporated by reference herein in its entirety.

In one embodiment, a siNA molecule of the invention is complexed with membrane disruptive agents such as those described in U.S. Patent Application

Publication No. 20010007666, incorporated by reference herein in its entirety including the drawings. In another embodiment, the membrane disruptive agent or agents and the siNA molecule are also complexed with a cationic lipid or helper lipid molecule, such as those lipids described in U.S. Patent No. 6,235,310, incorporated by reference herein in its entirety including the drawings.

In one embodiment, a siNA molecule of the invention is complexed with delivery systems as described in U.S. Patent Application Publication No. 2003077829 and International PCT Publication Nos. WO 00/03683 and WO 02/087541, all incorporated by reference herein in their entirety including the drawings.

In one embodiment, delivery systems of the invention include, for example, aqueous and nonaqueous gels, creams, multiple emulsions, microemulsions, liposomes, ointments, aqueous and nonaqueous solutions, lotions, aerosols, hydrocarbon bases and powders, and can contain excipients such as solubilizers, permeation enhancers (e.g., fatty acids, fatty acid esters, fatty alcohols and amino acids), and hydrophilic polymers (e.g., polycarbophil and polyvinylpyrolidone). In one embodiment, the pharmaceutically acceptable carrier is a liposome or a transdermal enhancer. Examples of liposomes which can be used in this invention include the following: (1) CellFectin, 1:1.5 (M/M) liposome formulation of the cationic lipid N,NI,NII,NIII-tetramethyl-N,NI,NII,NIII-tetrapalmit-y-spermine and dioleoyl phosphatidylethanolamine (DOPE) (GIBCO BRL); (2) Cytofectin GSV, 2:1 (M/M) liposome formulation of a cationic lipid and DOPE (Glen Research); (3) DOTAP (N-[1-(2,3-dioleoyloxy)-N,N,N-tri-methyl-ammoniummethylsulfate) (Boehringer Manheim); and (4) Lipofectamine, 3:1 (M/M) liposome formulation of the polycationic lipid DOSPA and the neutral lipid DOPE (GIBCO BRL).

In one embodiment, delivery systems of the invention include patches, tablets, suppositories, pessaries, gels and creams, and can contain excipients such as solubilizers and enhancers (e.g., propylene glycol, bile salts and amino acids), and other vehicles (e.g., polyethylene glycol, fatty acid esters and derivatives, and hydrophilic polymers such as hydroxypropylmethylcellulose and hyaluronic acid).

In one embodiment, siNA molecules of the invention are formulated or complexed with polyethylenimine (e.g., linear or branched PEI) and/or polyethylenimine derivatives, including for example grafted PEIs such as galactose PEI, cholesterol PEI,

antibody derivatized PEI, and polyethylene glycol PEI (PEG-PEI) derivatives thereof (see for example Ogris *et al.*, 2001, *AAPA PharmSci*, 3, 1-11; Furgeson et al., 2003, Bioconjugate Chem., 14, 840-847; Kunath et al., 2002, Phramaceutical Research, 19, 810-817; Choi et al., 2001, Bull. Korean Chem. Soc., 22, 46-52; Bettinger et al., 1999, Bioconjugate Chem., 10, 558-561; Peterson et al., 2002, Bioconjugate Chem., 13, 845-854; Erbacher et al., 1999, Journal of Gene Medicine Preprint, 1, 1-18; Godbey et al., 1999., PNAS USA, 96, 5177-5181; Godbey et al., 1999, Journal of Controlled Release, 60, 149-160; Diebold et al., 1999, Journal of Biological Chemistry, 274, 19087-19094; Thomas and Klibanov, 2002, PNAS USA, 99, 14640-14645; and Sagara, US 6,586,524, incorporated by reference herein.

In one embodiment, a siNA molecule of the invention comprises a bioconjugate, for example a nucleic acid conjugate as described in Vargeese et al., USSN 10/427,160, filed April 30, 2003; US 6,528,631; US 6,335,434; US 6, 235,886; US 6,153,737; US 5,214,136; US 5,138,045, all incorporated by reference herein.

Thus, the invention features a pharmaceutical composition comprising one or more nucleic acid(s) of the invention in an acceptable carrier, such as a stabilizer, buffer, and the like. The polynucleotides of the invention can be administered (*e.g.*, RNA, DNA or protein) and introduced to a subject by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention can also be formulated and used as creams, gels, sprays, oils and other suitable compositions for topical, dermal, or transdermal administration as is known in the art.

The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, *e.g.*, acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, *e.g.*, systemic or local administration, into a cell or subject, including for example a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such

forms should not prevent the composition or formulation from reaching a target cell (*i.e.*, a cell to which the negatively charged nucleic acid is desirable for delivery). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect.

In one embodiment, siNA molecules of the invention are administered to a subject by systemic administration in a pharmaceutically acceptable composition or formulation. By "systemic administration" is meant *in vivo* systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes that lead to systemic absorption include, without limitation: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes exposes the siNA molecules of the invention to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation that can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach can provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells.

By "pharmaceutically acceptable formulation" or "pharmaceutically acceptable composition" is meant, a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the instant invention include: P-glycoprotein inhibitors (such as Pluronic P85),; biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery (Emerich, DF *et al*, 1999, *Cell Transplant*, 8, 47-58); and loaded nanoparticles, such as those made of polybutylcyanoacrylate. Other non-limiting examples of delivery strategies for the nucleic acid molecules of the instant invention include material described in Boado *et al.*, 1998, *J. Pharm. Sci.*, 87, 1308-1315; Tyler *et al.*, 1999, *FEBS Lett.*, 421, 280-284;

Pardridge *et al.*, 1995, *PNAS USA.*, 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery Rev.*, 15, 73-107; Aldrian-Herrada *et al.*, 1998, *Nucleic Acids Res.*, 26, 4910-4916; and Tyler *et al.*, 1999, *PNAS USA.*, 96, 7053-7058.

The invention also features the use of the composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic *et al. Chem. Rev.* 1995, 95, 2601-2627; Ishiwata *et al., Chem. Pharm. Bull.* 1995, 43, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic *et al., Science* 1995, 267, 1275-1276; Oku *et al.*,1995, *Biochim. Biophys. Acta*, 1238, 86-90). The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu *et al., J. Biol. Chem.* 1995, 42, 24864-24870; Choi *et al.*, International PCT Publication No. WO 96/10391; Ansell *et al.*, International PCT Publication No. WO 96/10390; Holland *et al.*, International PCT Publication No. WO 96/10392). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

The present invention also includes compositions prepared for storage or administration that include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985), hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors that those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

The nucleic acid molecules of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and/or vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (*e.g.*, intravenous), intramuscular, or intrathecal injection or infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be, for example, inert diluents; such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia; and lubricating agents, for example magnesium

stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in a mixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The nucleic acid molecules of the invention can also be administered in the form of suppositories, *e.g.*, for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the

rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per subject per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the host treated and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

It is understood that the specific dose level for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The nucleic acid molecules of the present invention can also be administered to a subject in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

In one embodiment, the invention comprises compositions suitable for administering nucleic acid molecules of the invention to specific cell types. For example, the asialoglycoprotein receptor (ASGPr) (Wu and Wu, 1987, *J. Biol. Chem.* 262, 4429-4432) is unique to hepatocytes and binds branched galactose-terminal

glycoproteins, such as asialoorosomucoid (ASOR). In another example, the folate receptor is overexpressed in many cancer cells. Binding of such glycoproteins, synthetic glycoconjugates, or folates to the receptor takes place with an affinity that strongly depends on the degree of branching of the oligosaccharide chain, for example, triatennary structures are bound with greater affinity than biatenarry or monoatennary chains (Baenziger and Fiete, 1980, *Cell,* 22, 611-620; Connolly *et al.,* 1982, *J. Biol. Chem.,* 257, 939-945). Lee and Lee, 1987, *Glycoconjugate J.*, 4, 317-328, obtained this high specificity through the use of N-acetyl-D-galactosamine as the carbohydrate moiety, which has higher affinity for the receptor, compared to galactose. This "clustering effect" has also been described for the binding and uptake of mannosyl-terminating glycoproteins or glycoconjugates (Ponpipom *et al.,* 1981, *J. Med. Chem.,* 24, 1388-1395). The use of galactose, galactosamine, or folate based conjugates to transport exogenous compounds across cell membranes can provide a targeted delivery approach to, for example, the treatment of liver disease, cancers of the liver, or other cancers. The use of bioconjugates can also provide a reduction in the required dose of therapeutic compounds required for treatment. Furthermore, therapeutic bioavailability, pharmacodynamics, and pharmacokinetic parameters can be modulated through the use of nucleic acid bioconjugates of the invention. Non-limiting examples of such bioconjugates are described in Vargeese *et al.,* USSN 10/201,394, filed August 13, 2001; and Matulic-Adamic *et al.,* USSN 60/362,016, filed March 6, 2002. Alternatively, certain siNA molecules of the instant invention can be expressed within cells from eukaryotic promoters (*e.g.,* Izant and Weintraub, 1985, *Science,* 229, 345; McGarry and Lindquist, 1986, *Proc. Natl. Acad. Sci.*, USA 83, 399; Scanlon *et al.,* 1991, *Proc. Natl. Acad. Sci. USA,* 88, 10591-5; Kashani-Sabet *et al.,* 1992, *Antisense Res. Dev.*, 2, 3-15; Dropulic *et al.,* 1992, *J. Virol.,* 66, 1432-41; Weerasinghe *et al.,* 1991, *J. Virol.,* 65, 5531-4; Ojwang *et al.,* 1992, *Proc. Natl. Acad. Sci. USA*, 89, 10802-6; Chen *et al.,* 1992, *Nucleic Acids Res.*, 20, 4581-9; Sarver *et al.,* 1990 *Science,* 247, 1222-1225; Thompson *et al.,* 1995, *Nucleic Acids Res.*, 23, 2259; Good *et al.,* 1997, *Gene Therapy,* 4, 45. Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by a enzymatic nucleic acid (Draper *et al.,* PCT WO 93/23569, and Sullivan *et al.,* PCT WO 94/02595; Ohkawa *et al.,* 1992, *Nucleic Acids Symp. Ser.,* 27, 15-6; Taira *et al.,* 1991, *Nucleic*

Acids Res., 19, 5125-30; Ventura *et al.*, 1993, *Nucleic Acids Res.*, 21, 3249-55; Chowrira *et al.*, 1994, *J. Biol. Chem.*, 269, 25856.

In another aspect of the invention, RNA molecules of the present invention can be expressed from transcription units (see for example Couture *et al.*, 1996, *TIG.*, 12, 510) inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. In another embodiment, pol III based constructs are used to express nucleic acid molecules of the invention (see for example Thompson, U.S. Pats. Nos. 5,902,880 and 6,146,886). The recombinant vectors capable of expressing the siNA molecules can be delivered as described above, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of nucleic acid molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecule interacts with the target mRNA and generates an RNAi response. Delivery of siNA molecule expressing vectors can be systemic, such as by intravenous or intra-muscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell (for a review see Couture *et al.*, 1996, *TIG.*, 12, 510).

In one aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the instant invention. The expression vector can encode one or both strands of a siNA duplex, or a single self-complementary strand that self hybridizes into a siNA duplex. The nucleic acid sequences encoding the siNA molecules of the instant invention can be operably linked in a manner that allows expression of the siNA molecule (see for example Paul *et al.*, 2002, *Nature Biotechnology*, 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology*, 19, 497; Lee *et al.*, 2002, *Nature Biotechnology*, 19, 500; and Novina *et al.*, 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725).

In another aspect, the invention features an expression vector comprising: a) a transcription initiation region (*e.g.*, eukaryotic pol I, II or III initiation region); b) a transcription termination region (*e.g.*, eukaryotic pol I, II or III termination region); and c) a nucleic acid sequence encoding at least one of the siNA molecules of the instant

invention, wherein said sequence is operably linked to said initiation region and said termination region in a manner that allows expression and/or delivery of the siNA molecule. The vector can optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the siNA of the invention; and/or an intron (intervening sequences).

Transcription of the siNA molecule sequences can be driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters are expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type depends on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, *Proc. Natl. Acad. Sci. U S A*, 87, 6743-7; Gao and Huang 1993, *Nucleic Acids Res.*, 21, 2867-72; Lieber *et al.*, 1993, *Methods Enzymol.*, 217, 47-66; Zhou *et al.*, 1990, *Mol. Cell. Biol.*, 10, 4529-37). Several investigators have demonstrated that nucleic acid molecules expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. U S A*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Yu *et al.*, 1993, *Proc. Natl. Acad. Sci. U S A*, 90, 6340-4; L'Huillier *et al.*, 1992, *EMBO J.*, 11, 4411-8; Lisziewicz *et al.*, 1993, *Proc. Natl. Acad. Sci. U. S. A*, 90, 8000-4; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Sullenger & Cech, 1993, *Science*, 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as siNA in cells (Thompson *et al.*, *supra*; Couture and Stinchcomb, *1996, supra*; Noonberg *et al.*, 1994, *Nucleic Acid Res.*, 22, 2830; Noonberg *et al.*, U.S. Pat. No. 5,624,803; Good *et al.*, 1997, *Gene Ther.*, 4, 45; Beigelman *et al.*, International PCT Publication No. WO 96/18736. The above siNA transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, *1996, supra)*.

In another aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the siNA molecules of the invention in a manner that allows expression of that siNA molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; and c) a nucleic acid sequence encoding at least one strand of the siNA molecule, wherein the sequence is operably linked to the initiation region and the termination region in a manner that allows expression and/or delivery of the siNA molecule.

In another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; and d) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the open reading frame and the termination region in a manner that allows expression and/or delivery of the siNA molecule. In yet another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; and d) a nucleic acid sequence encoding at least one siNA molecule, wherein the sequence is operably linked to the initiation region, the intron and the termination region in a manner which allows expression and/or delivery of the nucleic acid molecule.

In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; and e) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the intron, the open reading frame and the termination region in a manner which allows expression and/or delivery of the siNA molecule.

XIAP biology and biochemistry

Apoptosis is a physiological cell death process that is important in the development, homeostasis, and immune defense of multicellular animals. The inhibitor of apoptosis (IAP) gene family encodes a group of structurally related proteins that have the ability to suppress apoptotic cell death by binding to and inhibiting caspases (Lotocki *et al.*, 2002, *IUBMB Life*, 54(5), 231 and Salvesen *et al.*, 2002, *Nature Reviews*

Molecular Cell Biology, 3, 401). Caspses are cysteine proteases with a substrate preference for aspartic acid and are the key effectors of apoptosis (Verhagen *et al.*, 2001 *GenomeBiology*, 2). All IAP's are BIR (baculovirus IAP repeat) containing proteins and BIRs are essential for the anti-apoptotic properties of the IAP's because they have been attributed to the binding and inhibition of caspases (Salvesen *et al.*, supra). IAP's can be induced by the transcription factor NF-KB or v-Rel, and HIAP1 and HIAP2 can activiate NF-KB (LaCasse *et al.*, 1998, *Oncogene*, 17(25), 3247).

XIAP (X-linked inhibitor of apoptosis protein) is a 57-kDA protein (Salvesen *et al.*, supra). XIAP is also a mammalian inhibitor of apoptosis protein and is a suppressor of apoptotic cell death. XIAP blocks the mitochondrial death pathway by binding directly to certain initiator and effector caspases. (Li *et al.*, 2003 *Hebei Daxue Xuebao, Ziran Kexueban* 23, 100). However, XIAP mutants that cannot bind caspases can still inhibit apoptosis (Salvesen *et al.*, supra). When cells are infected by a virus, such as cancer, XIAP inhibits the apoptosis that would occur and the cancer cells continue inappropriate proliferation. Other IAPs (inhibitor of apoptosis proteins) including HIAP1, HIAP2 (human inhibitor or apoptosis 1 and 2), and NAIP (neuronal apoptosis inhibitor protein) can also suppress apoptosis.

Because XIAP and other IAP's, including HIAP1, HIAP2, and NAIP, are inhibitors of apoptosis, modulation of IAP gene expression using RNA interfernce medicated by short interfering nucleic acids represents a novel treatment approach for cancer and other proliferative diseases and conditions where the regulation of apoptosis is lost.

Examples:

The following are non-limiting examples showing the selection, isolation, synthesis and activity of nucleic acids of the instant invention.

Example 1: Tandem synthesis of siNA constructs

Exemplary siNA molecules of the invention are synthesized in tandem using a cleavable linker, for example, a succinyl-based linker. Tandem synthesis as described herein is followed by a one-step purification process that provides RNAi molecules in high yield. This approach is highly amenable to siNA synthesis in support of high

throughput RNAi screening, and can be readily adapted to multi-column or multi-well synthesis platforms.

After completing a tandem synthesis of a siNA oligo and its complement in which the 5'-terminal dimethoxytrityl (5'-O-DMT) group remains intact (trityl on synthesis), the oligonucleotides are deprotected as described above. Following deprotection, the siNA sequence strands are allowed to spontaneously hybridize. This hybridization yields a duplex in which one strand has retained the 5'-O-DMT group while the complementary strand comprises a terminal 5'-hydroxyl. The newly formed duplex behaves as a single molecule during routine solid-phase extraction purification (Trityl-On purification) even though only one molecule has a dimethoxytrityl group. Because the strands form a stable duplex, this dimethoxytrityl group (or an equivalent group, such as other trityl groups or other hydrophobic moieties) is all that is required to purify the pair of oligos, for example, by using a C18 cartridge.

Standard phosphoramidite synthesis chemistry is used up to the point of introducing a tandem linker, such as an inverted deoxy abasic succinate or glyceryl succinate linker (see **Figure 1**) or an equivalent cleavable linker. A non-limiting example of linker coupling conditions that can be used includes a hindered base such as diisopropylethylamine (DIPA) and/or DMAP in the presence of an activator reagent such as Bromotripyrrolidinophosphoniumhexaflurorophosphate (PyBrOP). After the linker is coupled, standard synthesis chemistry is utilized to complete synthesis of the second sequence leaving the terminal the 5'-O-DMT intact. Following synthesis, the resulting oligonucleotide is deprotected according to the procedures described herein and quenched with a suitable buffer, for example with 50mM NaOAc or 1.5M $NH_4H_2CO_3$.

Purification of the siNA duplex can be readily accomplished using solid phase extraction, for example, using a Waters C18 SepPak 1g cartridge conditioned with 1 column volume (CV) of acetonitrile, 2 CV H2O, and 2 CV 50mM NaOAc. The sample is loaded and then washed with 1 CV H2O or 50mM NaOAc. Failure sequences are eluted with 1 CV 14% ACN (Aqueous with 50mM NaOAc and 50mM NaCl). The column is then washed, for example with 1 CV H2O followed by on-column detritylation, for example by passing 1 CV of 1% aqueous trifluoroacetic acid (TFA) over the column, then adding a second CV of 1% aqueous TFA to the column and

allowing to stand for approximately 10 minutes. The remaining TFA solution is removed and the column washed with H20 followed by 1 CV 1M NaCl and additional H2O. The siNA duplex product is then eluted, for example, using 1 CV 20% aqueous CAN.

Figure 2 provides an example of MALDI-TOF mass spectrometry analysis of a purified siNA construct in which each peak corresponds to the calculated mass of an individual siNA strand of the siNA duplex. The same purified siNA provides three peaks when analyzed by capillary gel electrophoresis (CGE), one peak presumably corresponding to the duplex siNA, and two peaks presumably corresponding to the separate siNA sequence strands. Ion exchange HPLC analysis of the same siNA contract only shows a single peak. Testing of the purified siNA construct using a luciferase reporter assay described below demonstrated the same RNAi activity compared to siNA constructs generated from separately synthesized oligonucleotide sequence strands.

Example 2: Identification of potential siNA target sites in any RNA sequence

The sequence of an RNA target of interest, such as a viral or human mRNA transcript, is screened for target sites, for example by using a computer folding algorithm. In a non-limiting example, the sequence of a gene or RNA gene transcript derived from a database, such as Genbank, is used to generate siNA targets having complementarity to the target. Such sequences can be obtained from a database, or can be determined experimentally as known in the art. Target sites that are known, for example, those target sites determined to be effective target sites based on studies with other nucleic acid molecules, for example ribozymes or antisense, or those targets known to be associated with a disease or condition such as those sites containing mutations or deletions, can be used to design siNA molecules targeting those sites. Various parameters can be used to determine which sites are the most suitable target sites within the target RNA sequence. These parameters include but are not limited to secondary or tertiary RNA structure, the nucleotide base composition of the target sequence, the degree of homology between various regions of the target sequence, or the relative position of the target sequence within the RNA transcript. Based on these determinations, any number of target sites within the RNA transcript can be chosen to screen siNA molecules for efficacy, for example by using *in vitro* RNA cleavage assays,

cell culture, or animal models. In a non-limiting example, anywhere from 1 to 1000 target sites are chosen within the transcript based on the size of the siNA construct to be used. High throughput screening assays can be developed for screening siNA molecules using methods known in the art, such as with multi-well or multi-plate assays to determine efficient reduction in target gene expression.

Example 3: Selection of siNA molecule target sites in a RNA

The following non-limiting steps can be used to carry out the selection of siNAs targeting a given gene sequence or transcript.

1. The target sequence is parsed *in silico* into a list of all fragments or subsequences of a particular length, for example 23 nucleotide fragments, contained within the target sequence. This step is typically carried out using a custom Perl script, but commercial sequence analysis programs such as Oligo, MacVector, or the GCG Wisconsin Package can be employed as well.

2. In some instances the siNAs correspond to more than one target sequence; such would be the case for example in targeting different transcripts of the same gene, targeting different transcripts of more than one gene, or for targeting both the human gene and an animal homolog. In this case, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find matching sequences in each list. The subsequences are then ranked according to the number of target sequences that contain the given subsequence; the goal is to find subsequences that are present in most or all of the target sequences. Alternately, the ranking can identify subsequences that are unique to a target sequence, such as a mutant target sequence. Such an approach would enable the use of siNA to target specifically the mutant sequence and not effect the expression of the normal sequence.

3. In some instances the siNA subsequences are absent in one or more sequences while present in the desired target sequence; such would be the case if the siNA targets a gene with a paralogous family member that is to remain untargeted. As in case 2 above, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find sequences that are present in the target gene but are absent in the untargeted paralog.

4. The ranked siNA subsequences can be further analyzed and ranked according to GC content. A preference can be given to sites containing 30-70% GC, with a further preference to sites containing 40-60% GC.

5. The ranked siNA subsequences can be further analyzed and ranked according to self-folding and internal hairpins. Weaker internal folds are preferred; strong hairpin structures are to be avoided.

6. The ranked siNA subsequences can be further analyzed and ranked according to whether they have runs of GGG or CCC in the sequence. GGG (or even more Gs) in either strand can make oligonucleotide synthesis problematic and can potentially interfere with RNAi activity, so it is avoided whenever better sequences are available. CCC is searched in the target strand because that will place GGG in the antisense strand.

7. The ranked siNA subsequences can be further analyzed and ranked according to whether they have the dinucleotide UU (uridine dinucleotide) on the 3'-end of the sequence, and/or AA on the 5'-end of the sequence (to yield 3' UU on the antisense sequence). These sequences allow one to design siNA molecules with terminal TT thymidine dinucleotides.

8. Four or five target sites are chosen from the ranked list of subsequences as described above. For example, in subsequences having 23 nucleotides, the right 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the upper (sense) strand of the siNA duplex, while the reverse complement of the left 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the lower (antisense) strand of the siNA duplex (see **Tables II and III**). If terminal TT residues are desired for the sequence (as described in paragraph 7), then the two 3' terminal nucleotides of both the sense and antisense strands are replaced by TT prior to synthesizing the oligos.

9. The siNA molecules are screened in an *in vitro*, cell culture or animal model system to identify the most active siNA molecule or the most preferred target site within the target RNA sequence.

Other design considerations can be used when selecting target nucleic acid sequences, see, for example, Reynolds *et al.*, 2004, *Nature Biotechnology Advanced*

Online Publication, 1 February 2004, doi:10.1038/nbt936 and Ui-Tei et al., 2004, Nucleic Acids Research, 32, doi:10.1093/nar/gkh247.

In an alternate approach, a pool of siNA constructs specific to a XIAP target sequence is used to screen for target sites in cells expressing XIAP RNA, such as cultured human T cells. The general strategy used in this approach is shown in **Figure 9.** A non-limiting example of such is a pool comprising sequences having any of SEQ ID NOS 1-1056. Cells expressing XIAP (e.g., cultured human T cells) are transfected with the pool of siNA constructs and cells that demonstrate a phenotype associated with XIAP inhibition are sorted. The pool of siNA constructs can be expressed from transcription cassettes inserted into appropriate vectors (see for example **Figure 7** and **Figure 8**). The siNA from cells demonstrating a positive phenotypic change (e.g., decreased proliferation, decreased XIAP mRNA levels or decreased XIAP protein expression), are sequenced to determine the most suitable target site(s) within the target XIAP RNA sequence.

Example 4: XIAP targeted siNA design

siNA target sites were chosen by analyzing sequences of the XIAP RNA target and optionally prioritizing the target sites on the basis of folding (structure of any given sequence analyzed to determine siNA accessibility to the target), by using a library of siNA molecules as described in Example 3, or alternately by using an *in vitro* siNA system as described in Example 6 herein. siNA molecules were designed that could bind each target and are optionally individually analyzed by computer folding to assess whether the siNA molecule can interact with the target sequence. Varying the length of the siNA molecules can be chosen to optimize activity. Generally, a sufficient number of complementary nucleotide bases are chosen to bind to, or otherwise interact with, the target RNA, but the degree of complementarity can be modulated to accommodate siNA duplexes or varying length or base composition. By using such methodologies, siNA molecules can be designed to target sites within any known RNA sequence, for example those RNA sequences corresponding to the any gene transcript.

Chemically modified siNA constructs are designed to provide nuclease stability for systemic administration in vivo and/or improved pharmacokinetic, localization, and delivery properties while preserving the ability to mediate RNAi activity. Chemical

modifications as described herein are introduced synthetically using synthetic methods described herein and those generally known in the art. The synthetic siNA constructs are then assayed for nuclease stability in serum and/or cellular/tissue extracts (e.g. liver extracts). The synthetic siNA constructs are also tested in parallel for RNAi activity using an appropriate assay, such as a luciferase reporter assay as described herein or another suitable assay that can quantity RNAi activity. Synthetic siNA constructs that possess both nuclease stability and RNAi activity can be further modified and re-evaluated in stability and activity assays. The chemical modifications of the stabilized active siNA constructs can then be applied to any siNA sequence targeting any chosen RNA and used, for example, in target screening assays to pick lead siNA compounds for therapeutic development (see for example **Figure 11**).

Example 5: Chemical Synthesis and Purification of siNA

siNA molecules can be designed to interact with various sites in the RNA message, for example, target sequences within the RNA sequences described herein. The sequence of one strand of the siNA molecule(s) is complementary to the target site sequences described above. The siNA molecules can be chemically synthesized using methods described herein. Inactive siNA molecules that are used as control sequences can be synthesized by scrambling the sequence of the siNA molecules such that it is not complementary to the target sequence. Generally, siNA constructs can by synthesized using solid phase oligonucleotide synthesis methods as described herein (see for example Usman *et al.*, US Patent Nos. 5,804,683; 5,831,071; 5,998,203; 6,117,657; 6,353,098; 6,362,323; 6,437,117; 6,469,158; Scaringe *et al.*, US Patent Nos. 6,111,086; 6,008,400; 6,111,086 all incorporated by reference herein in their entirety).

In a non-limiting example, RNA oligonucleotides are synthesized in a stepwise fashion using the phosphoramidite chemistry as is known in the art. Standard phosphoramidite chemistry involves the use of nucleosides comprising any of 5'-O-dimethoxytrityl, 2'-O-tert-butyldimethylsilyl, 3'-O-2-Cyanoethyl N,N-diisopropylphosphoroamidite groups, and exocyclic amine protecting groups (e.g. N6-benzoyl adenosine, N4 acetyl cytidine, and N2-isobutyryl guanosine). Alternately, 2'-O-Silyl Ethers can be used in conjunction with acid-labile 2'-O-orthoester protecting groups in the synthesis of RNA as described by Scaringe *supra*. Differing 2' chemistries can require different

protecting groups, for example 2'-deoxy-2'-amino nucleosides can utilize N-phthaloyl protection as described by Usman *et al.*, US Patent 5,631,360, incorporated by reference herein in its entirety).

During solid phase synthesis, each nucleotide is added sequentially (3′- to 5′-direction) to the solid support-bound oligonucleotide. The first nucleoside at the 3′-end of the chain is covalently attached to a solid support (e.g., controlled pore glass or polystyrene) using various linkers. The nucleotide precursor, a ribonucleoside phosphoramidite, and activator are combined resulting in the coupling of the second nucleoside phosphoramidite onto the 5′-end of the first nucleoside. The support is then washed and any unreacted 5′-hydroxyl groups are capped with a capping reagent such as acetic anhydride to yield inactive 5′-acetyl moieties. The trivalent phosphorus linkage is then oxidized to a more stable phosphate linkage. At the end of the nucleotide addition cycle, the 5′-O-protecting group is cleaved under suitable conditions (e.g., acidic conditions for trityl-based groups and Fluoride for silyl-based groups). The cycle is repeated for each subsequent nucleotide.

Modification of synthesis conditions can be used to optimize coupling efficiency, for example by using differing coupling times, differing reagent/phosphoramidite concentrations, differing contact times, differing solid supports and solid support linker chemistries depending on the particular chemical composition of the siNA to be synthesized. Deprotection and purification of the siNA can be performed as is generally described in Usman *et al.*, US 5,831,071, US 6,353,098, US 6,437,117, and Bellon *et al.*, US 6,054,576, US 6,162,909, US 6,303,773, or Scaringe *supra,* incorporated by reference herein in their entireties. Additionally, deprotection conditions can be modified to provide the best possible yield and purity of siNA constructs. For example, applicant has observed that oligonucleotides comprising 2'-deoxy-2'-fluoro nucleotides can degrade under inappropriate deprotection conditions. Such oligonucleotides are deprotected using aqueous methylamine at about 35°C for 30 minutes. If the 2'-deoxy-2'-fluoro containing oligonucleotide also comprises ribonucleotides, after deprotection with aqueous methylamine at about 35°C for 30 minutes, TEA-HF is added and the reaction maintained at about 65°C for an additional 15 minutes.

Example 6: RNAi *in vitro* assay to assess siNA activity

An *in vitro* assay that recapitulates RNAi in a cell-free system is used to evaluate siNA constructs targeting XIAP RNA targets. The assay comprises the system described by Tuschl *et al.*, 1999, *Genes and Development*, 13, 3191-3197 and Zamore *et al.*, 2000, *Cell*, 101, 25-33 adapted for use with XIAP target RNA. A Drosophila extract derived from syncytial blastoderm is used to reconstitute RNAi activity *in vitro*. Target RNA is generated via *in vitro* transcription from an appropriate XIAP expressing plasmid using T7 RNA polymerase or via chemical synthesis as described herein. Sense and antisense siNA strands (for example 20 uM each) are annealed by incubation in buffer (such as 100 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 2 mM magnesium acetate) for 1 minute at 90°C followed by 1 hour at 37°C , then diluted in lysis buffer (for example 100 mM potassium acetate, 30 mM HEPES-KOH at pH 7.4, 2mM magnesium acetate). Annealing can be monitored by gel electrophoresis on an agarose gel in TBE buffer and stained with ethidium bromide. The Drosophila lysate is prepared using zero to two-hour-old embryos from Oregon R flies collected on yeasted molasses agar that are dechorionated and lysed. The lysate is centrifuged and the supernatant isolated. The assay comprises a reaction mixture containing 50% lysate [vol/vol], RNA (10-50 pM final concentration), and 10% [vol/vol] lysis buffer containing siNA (10 nM final concentration). The reaction mixture also contains 10 mM creatine phosphate, 10 ug/ml creatine phosphokinase, 100 um GTP, 100 uM UTP, 100 uM CTP, 500 uM ATP, 5 mM DTT, 0.1 U/uL RNasin (Promega), and 100 uM of each amino acid. The final concentration of potassium acetate is adjusted to 100 mM. The reactions are pre-assembled on ice and preincubated at 25° C for 10 minutes before adding RNA, then incubated at 25° C for an additional 60 minutes. Reactions are quenched with 4 volumes of 1.25 x Passive Lysis Buffer (Promega). Target RNA cleavage is assayed by RT-PCR analysis or other methods known in the art and are compared to control reactions in which siNA is omitted from the reaction.

Alternately, internally-labeled target RNA for the assay is prepared by *in vitro* transcription in the presence of [alpha-^{32}P] CTP, passed over a G50 Sephadex column by spin chromatography and used as target RNA without further purification. Optionally, target RNA is 5'-^{32}P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed as described above and target RNA and the specific RNA cleavage products

generated by RNAi are visualized on an autoradiograph of a gel. The percentage of cleavage is determined by PHOSPHOR IMAGER® (autoradiography) quantitation of bands representing intact control RNA or RNA from control reactions without siNA and the cleavage products generated by the assay.

In one embodiment, this assay is used to determine target sites in the XIAP RNA target for siNA mediated RNAi cleavage, wherein a plurality of siNA constructs are screened for RNAi mediated cleavage of the XIAP RNA target, for example, by analyzing the assay reaction by electrophoresis of labeled target RNA, or by northern blotting, as well as by other methodology well known in the art.

Example 7: Nucleic acid inhibition of XIAP target RNA

siNA molecules targeted to the human XIAP RNA are designed and synthesized as described above. These nucleic acid molecules can be tested for cleavage activity *in vivo*, for example, using the following procedure. The target sequences and the nucleotide location within the XIAP RNA are given in **Tables II and III.**

Two formats are used to test the efficacy of siNAs targeting XIAP. First, the reagents are tested in cell culture using, for example, cultured human T-cells, A549 cells, or HeLa cells, to determine the extent of RNA and protein inhibition. siNA reagents (*e.g.*; see **Tables II and III**) are selected against the XIAP target as described herein. RNA inhibition is measured after delivery of these reagents by a suitable transfection agent to, for example, cultured human T-cells, A549 cells, or HeLa cells. Relative amounts of target RNA are measured versus actin using real-time PCR monitoring of amplification (*eg.*, ABI 7700 TAQMAN®). A comparison is made to a mixture of oligonucleotide sequences made to unrelated targets or to a randomized siNA control with the same overall length and chemistry, but randomly substituted at each position. Primary and secondary lead reagents are chosen for the target and optimization performed. After an optimal transfection agent concentration is chosen, a RNA time-course of inhibition is performed with the lead siNA molecule. In addition, a cell-plating format can be used to determine RNA inhibition.

Delivery of siNA to Cells

Cells (e.g., cultured human T-cells, A549 cells, or HeLa cells) are seeded, for example, at $1x10^5$ cells per well of a six-well dish in EGM-2 (BioWhittaker) the day before transfection. siNA (final concentration, for example 20nM) and cationic lipid (e.g., final concentration 2μg/ml) are complexed in EGM basal media (Bio Whittaker) at 37°C for 30 minutes in polystyrene tubes. Following vortexing, the complexed siNA is added to each well and incubated for the times indicated. For initial optimization experiments, cells are seeded, for example, at $1x10^3$ in 96 well plates and siNA complex added as described. Efficiency of delivery of siNA to cells is determined using a fluorescent siNA complexed with lipid. Cells in 6-well dishes are incubated with siNA for 24 hours, rinsed with PBS and fixed in 2% paraformaldehyde for 15 minutes at room temperature. Uptake of siNA is visualized using a fluorescent microscope.

TAQMAN® (real-time PCR monitoring of amplification) and Lightcycler quantification of mRNA

Total RNA is prepared from cells following siNA delivery, for example, using Qiagen RNA purification kits for 6-well or Rneasy extraction kits for 96-well assays. For TAQMAN® analysis (real-time PCR monitoring of amplification), dual-labeled probes are synthesized with the reporter dye, FAM or JOE, covalently linked at the 5'-end and the quencher dye TAMRA conjugated to the 3'-end. One-step RT-PCR amplifications are performed on, for example, an ABI PRISM 7700 Sequence Detector using 50 μl reactions consisting of 10 μl total RNA, 100 nM forward primer, 900 nM reverse primer, 100 nM probe, 1X TaqMan PCR reaction buffer (PE-Applied Biosystems), 5.5 mM $MgCl_2$, 300 μM each dATP, dCTP, dGTP, and dTTP, 10U RNase Inhibitor (Promega), 1.25U AMPLITAQ GOLD® (DNA polymerase) (PE-Applied Biosystems) and 10U M-MLV Reverse Transcriptase (Promega). The thermal cycling conditions can consist of 30 minutes at 48°C, 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Quantitation of mRNA levels is determined relative to standards generated from serially diluted total cellular RNA (300, 100, 33, 11 ng/rxn) and normalizing to ß-actin or GAPDH mRNA in parallel TAQMAN® reactions (real-time PCR monitoring of amplification). For each gene of interest an upper and lower primer and a fluorescently labeled probe are designed. Real time incorporation of SYBR Green

I dye into a specific PCR product can be measured in glass capillary tubes using a lightcyler. A standard curve is generated for each primer pair using control cRNA. Values are represented as relative expression to GAPDH in each sample.

Western blotting

Nuclear extracts can be prepared using a standard micro preparation technique (see for example Andrews and Faller, 1991, *Nucleic Acids Research,* 19, 2499). Protein extracts from supernatants are prepared, for example using TCA precipitation. An equal volume of 20% TCA is added to the cell supernatant, incubated on ice for 1 hour and pelleted by centrifugation for 5 minutes. Pellets are washed in acetone, dried and resuspended in water. Cellular protein extracts are run on a 10% Bis-Tris NuPage (nuclear extracts) or 4-12% Tris-Glycine (supernatant extracts) polyacrylamide gel and transferred onto nitro-cellulose membranes. Non-specific binding can be blocked by incubation, for example, with 5% non-fat milk for 1 hour followed by primary antibody for 16 hour at 4°C. Following washes, the secondary antibody is applied, for example (1:10,000 dilution) for 1 hour at room temperature and the signal detected with SuperSignal reagent (Pierce).

Example 8: Animal Models useful to evaluate the down-regulation of XIAP gene expression

Cell Culture

There are numerous cell culture systems that can be used to analyze reduction of XIAP levels either directly or indirectly by measuring downstream effects. For example, HELA cells can be used in cell culture experiments to assess the efficacy of nucleic acid molecules of the invention. As such, cells treated with nucleic acid molecules of the invention (e.g., siNA) targeting XIAP RNA would be expected to have decreased XIAP expression capacity compared to matched control nucleic acid molecules having a scrambled or inactive sequence. In a non-limiting example, HELA cells are cultured and XIAP expression is quantified, for example by time-resolved immuno fluorometric assay. XIAP messenger-RNA expression is quantitated with RT-PCR in cultured cells. Untreated cells are compared to cells treated with siNA molecules transfected with a suitable reagent, for example a cationic lipid such as lipofectamine, and XIAP protein

and RNA levels are quantitated. Dose response assays are then performed to establish dose dependent inhibition of XIAP expression. In a non-limiting examlple, cell culture experiments are adapted to those experiments described in Korneluk et al., International PCT Publication No. WO 02/26968.

In several cell culture systems, cationic lipids have been shown to enhance the bioavailability of oligonucleotides to cells in culture (Bennet, *et al.*, 1992, *Mol. Pharmacology*, 41, 1023-1033). In one embodiment, siNA molecules of the invention are complexed with cationic lipids for cell culture experiments. siNA and cationic lipid mixtures are prepared in serum-free DMEM immediately prior to addition to the cells. DMEM plus additives are warmed to room temperature (about 20-25°C) and cationic lipid is added to the final desired concentration and the solution is vortexed briefly. siNA molecules are added to the final desired concentration and the solution is again vortexed briefly and incubated for 10 minutes at room temperature. In dose response experiments, the RNA/lipid complex is serially diluted into DMEM following the 10 minute incubation.

Animal Models

Evaluating the efficacy of anti-XIAP agents in animal models is an important prerequisite to human clinical trials. The role of XIAP has recently been investigated (Conte *et al.*, 2001, *Proc. Natl. Acad. Sci. USA* , 98, 5049) using engineered transgenic mice that over express a human XIAP transgene under the control of a T cell specific promoter, lck., to assess the effect of XIAP on T cell development. The investigators evaluated the ability of XIAP to rescue apoptotic-sensitive thymocytes from apoptotic triggers, such as C2 ceramide, UV radiation, and anti-Fas antibody. Investigators found that lck-XIAP thymocytes demonstrated reduced in vitro apoptosis, with only 20% cell death relative to untreated lck-xiap thymocytes over 18 hours when exposed to C2 ceramide exposure. The ability of XIAP to inhibit apoptotic pathways after exposure to UV radiation and a Fas death receptor (anti-Fas antibody) led to the finding that lck-XIAP thymoctyes were resistant to apoptosis, with apoptosis being reduced compared with wild-type thymocytes (Conte *et al.*, *supra*).

In addition, thymocytes were treated with dexamethasone or anti-CD3 antibody in vitro which triggers apoptosis of thymoctyes; however the lck-XIAP thymocytes

demonstrated enhanced resistance to apoptosis. Thymocytes of control mice and lck-xiap mice were also injected with anti-Fas antibody to test levels of apoptosis in vivo. The control mice thymocytes had extensive apoptotic death while the thymocytes of lck-XIAP mice had significantly less apoptosis. The resistance to apoptosis by lck-XIAP thymocytes was attributable to over expression of XIAP (Conte *et al.*, *supra*).

The animal model described by Conte *et al.*, *supra*, can be used to evaluate inhibition of XIAP expression and the increased regression of tumor growth after the transfer of conditioned T-cells in the presence of a XIAP blockade using siNA molecules of the invention. The improved clearance of tumors in mice can be associated with the XIAP blockade that improves apoptosis of disease infected cells. These results raise the possibility that manipulation of XIAP can be used toward therapeutic use in preventing and/or treating cancer and other proliferative conditions discussed herein in human subjects.

Example 9: RNAi mediated inhibition of XIAP expression

siNA constructs (**Table III**) are tested for efficacy in reducing XIAP RNA expression in, for example, human T-cells, A549 cells, or HeLa cells. Cells are plated approximately 24 hours before transfection in 96-well plates at 5,000-7,500 cells/well, 100 µl/well, such that at the time of transfection cells are 70-90% confluent. For transfection, annealed siNAs are mixed with the transfection reagent (Lipofectamine 2000, Invitrogen) in a volume of 50 µl/well and incubated for 20 minutes at room temperature. The siNA transfection mixtures are added to cells to give a final siNA concentration of 25 nM in a volume of 150 µl. Each siNA transfection mixture is added to 3 wells for triplicate siNA treatments. Cells are incubated at 37° for 24 hours in the continued presence of the siNA transfection mixture. At 24 hours, RNA is prepared from each well of treated cells. The supernatants with the transfection mixtures are first removed and discarded, then the cells are lysed and RNA prepared from each well. Target gene expression following treatment is evaluated by RT-PCR for the target gene and for a control gene (36B4, an RNA polymerase subunit) for normalization. The triplicate data is averaged and the standard deviations determined for each treatment. Normalized data are graphed and the percent reduction of target mRNA by active siNAs in comparison to their respective inverted control siNAs is determined.

In a non-limiting example, chemically modified siNA constructs (**Table III**) were tested for efficacy as described above in XIAP RNA expression in A549 cells. Active siNAs were evaluated compared to untreated cells, matched chemistry irrelevant controls (IC1, IC2), and a transfection control. Results are summarized in **Figure 22**. **Figure 22** shows results for chemically modified siNA constructs targeting various sites in XIAP mRNA. As shown in **Figure 22**, the active siNA constructs provide significant inhibition of XIAP gene expression in cell culture experiments as determined by levels of XIAP mRNA when compared to appropriate controls.

Example 10: Indications

The present body of knowledge in inhibitors of apoptosis research indicates the need for methods and compounds that can regulate XIAP HIAP1, HIAP2, and/or NAIP gene expression for research, diagnostic, and therapeutic use. As described herein, the nucleic acid molecules of the present invention can be used to treat cancer and other proliferative conditions such as ovarian cancer; cancers of non-lymphoid parenchymal organs including the heart, placenta, skeletal muscle and lung; breast cancer, cancers of the head and neck including various lymphomas such as mantle cell lymphoma, non-Hodgkins lymphoma, adenoma, squamous cell carcinoma, laryngeal carcinoma, cancers of the retina, cancers of the esophagus, multiple myeloma, melanoma, colorectal cancer, lung cancer, bladder cancer, prostate cancer, glioblastoma; and proliferative diseases and conditions such as restenosis and polycystic kidney disease, ocular disease; and any other indications that can respond to the level of a XIAP, HIAP1, HIAP2, and/or NAIP gene in a cell or tissue.

The use of radiation treatments and chemotherapeutics, such as Gemcytabine and cyclophosphamide, are non-limiting examples of chemotherapeutic agents that can be combined with or used in conjunction with the nucleic acid molecules (*e.g.* siNA molecules) of the instant invention. Those skilled in the art will recognize that other anti-cancer compounds and therapies can similarly be readily combined with the nucleic acid molecules of the instant invention (*e.g.* siNA molecules) and are hence within the scope of the instant invention. Such compounds and therapies are well known in the art (see for example *Cancer: Principles and Pranctice of Oncology*, Volumes 1 and 2, eds Devita, V.T., Hellman, S., and Rosenberg, S.A., J.B. Lippincott Company, Philadelphia,

USA; incorporated herein by reference) and include, without limitation, folates, antifolates, pyrimidine analogs, fluoropyrimidines, purine analogs, adenosine analogs, topoisomerase I inhibitors, anthrapyrazoles, retinoids, antibiotics, anthacyclins, platinum analogs, alkylating agents, nitrosoureas, plant derived compounds such as vinca alkaloids, epipodophyllotoxins, tyrosine kinase inhibitors, taxols, radiation therapy, surgery, nutritional supplements, gene therapy, radiotherapy, for example 3D-CRT, immunotoxin therapy, for example ricin, and monoclonal antibodies. Specific examples of chemotherapeutic compounds that can be combined with or used in conjuction with the nucleic acid molecules of the invention include, but are not limited to, Paclitaxel; Docetaxel; Methotrexate; Doxorubin; Edatrexate; Vinorelbine; Tomaxifen; Leucovorin; 5-fluoro uridine (5-FU); Ionotecan; Cisplatin; Carboplatin; Amsacrine; Cytarabine; Bleomycin; Mitomycin C; Dactinomycin; Mithramycin; Hexamethylmelamine; Dacarbazine; L-asperginase; Nitrogen mustard; Melphalan, Chlorambucil; Busulfan; Ifosfamide; 4-hydroperoxycyclophosphamide; Thiotepa; Irinotecan (CAMPTOSAR®, CPT-11, Camptothecin-11, Campto) Tamoxifen; Herceptin; IMC C225; ABX-EGF; and combinations thereof. The above list of compounds are non-limiting examples of compounds and/or methods that can be combined with or used in conjunction with the nucleic acid molecules (*e.g.* siNA) of the instant invention. Those skilled in the art will recognize that other drug compounds and therapies can similarly be readily combined with the nucleic acid molecules of the instant invention (e.g., siNA molecules) are hence within the scope of the instant invention.

Example 11: Diagnostic uses

The siNA molecules of the invention can be used in a variety of diagnostic applications, such as in the identification of molecular targets (e.g., RNA) in a variety of applications, for example, in clinical, industrial, environmental, agricultural and/or research settings. Such diagnostic use of siNA molecules involves utilizing reconstituted RNAi systems, for example, using cellular lysates or partially purified cellular lysates. siNA molecules of this invention can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of endogenous or exogenous, for example viral, RNA in a cell. The close relationship between siNA activity and the structure of the target RNA allows the detection of mutations in any region of the molecule, which alters the base-pairing and three-dimensional structure of

the target RNA. By using multiple siNA molecules described in this invention, one can map nucleotide changes, which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with siNA molecules can be used to inhibit gene expression and define the role of specified gene products in the progression of disease or infection. In this manner, other genetic targets can be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple siNA molecules targeted to different genes, siNA molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations siNA molecules and/or other chemical or biological molecules). Other *in vitro* uses of siNA molecules of this invention are well known in the art, and include detection of the presence of mRNAs associated with a disease, infection, or related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a siNA using standard methodologies, for example, fluorescence resonance emission transfer (FRET).

In a specific example, siNA molecules that cleave only wild-type or mutant forms of the target RNA are used for the assay. The first siNA molecules (*i.e.*, those that cleave only wild-type forms of target RNA) are used to identify wild-type RNA present in the sample and the second siNA molecules (*i.e.*, those that cleave only mutant forms of target RNA) are used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA are cleaved by both siNA molecules to demonstrate the relative siNA efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus, each analysis requires two siNA molecules, two substrates and one unknown sample, which is combined into six reactions. The presence of cleavage products is determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, disease related or infection related) is adequate to

establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels is adequate and decreases the cost of the initial diagnosis. Higher mutant form to wild-type ratios are correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims. The present invention teaches one skilled in the art to test various combinations and/or substitutions of chemical modifications described herein toward generating nucleic acid constructs with improved activity for mediating RNAi activity. Such improved activity can comprise improved stability, improved bioavailability, and/or improved activation of cellular responses mediating RNAi. Therefore, the specific embodiments described herein are not limiting and one skilled in the art can readily appreciate that specific combinations of the modifications described herein can be tested without undue experimentation toward identifying siNA molecules with improved RNAi activity.

The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either

of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

Table I: XIAP Accession Numbers

BIRC1

LOCUS NM_004536 6133 bp mRNA
linear PRI 05-APR-2003
DEFINITION Homo sapiens baculoviral IAP repeat-containing
1 (BIRC1), mRNA.
ACCESSION NM_004536

BIRC2

LOCUS NM_001166 3496 bp mRNA
linear PRI 03-APR-2003
DEFINITION Homo sapiens baculoviral IAP repeat-containing
2 (BIRC2), mRNA.
ACCESSION NM_001166

BIRC3

LOCUS NM_001165 3165 bp mRNA
linear PRI 03-APR-2003
DEFINITION Homo sapiens baculoviral IAP repeat-containing
3 (BIRC3), mRNA.
ACCESSION NM_001165

BIRC4

LOCUS NM_001167 8413 bp mRNA
linear PRI 11-JUL-2003
DEFINITION Homo sapiens baculoviral IAP repeat-containing
4 (BIRC4), mRNA.
ACCESSION NM_001167

BIRC5

LOCUS NM_001168 1619 bp mRNA
linear PRI 03-APR-2003
DEFINITION Homo sapiens baculoviral IAP repeat-containing
5 (survivin)
(BIRC5), mRNA.
ACCESSION NM_001168

BIRC6

LOCUS NM_016252 14490 bp mRNA
linear PRI 06-APR-2003
DEFINITION Homo sapiens baculoviral IAP repeat-containing
6 (apollon) (BIRC6),
mRNA.
ACCESSION NM_016252

BIRC7-1

LOCUS NM_139317 1322 bp mRNA
linear PRI 06-APR-2003
DEFINITION Homo sapiens baculoviral IAP repeat-containing
7 (livin) (BIRC7),
transcript variant 1, mRNA.
ACCESSION NM_139317

BIRC7-2

LOCUS NM_022161 1268 bp mRNA
linear PRI 06-APR-2003
DEFINITION Homo sapiens baculoviral IAP repeat-containing
7 (livin) (BIRC7), transcript variant 2, mRNA.
ACCESSION NM_022161

BIRC8

LOCUS NM_033341 2032 bp mRNA
linear PRI 06-APR-2003
DEFINITION Homo sapiens baculoviral IAP repeat-containing
8 (BIRC8), mRNA.
ACCESSION NM_033341
VERSION NM_033341.2 GI:16974127

TABLE II: XIAP/BIRC4 siNA AND TARGET SEQUENCES

XIAP BIRC4|NM_001167.2

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3	UCCAGAUUGGGGCUCGGGC	1	3	UCCAGAUUGGGGCUCGGGC	1	21	GCCCGAGCCCCAAUCUGGA	468
21	CCGCGCCUCCUCCGGGACC	2	21	CCGCGCCUCCUCCGGGACC	2	39	GGUCCCGGAGGAGGCGCGG	469
39	CCUCCCCUUGGACCGAGCC	3	39	CCUCCCCUUGGACCGAGCC	3	57	GGCUCGGUCCAAGGGGAGG	470
57	CGAUCGCCGCGGGGCAGUU	4	57	CGAUCGCCGCGGGGCAGUU	4	75	AACUGCCCCGCGGCGAUCG	471
75	UCGGGCCGGCUGUCCUGGC	5	75	UCGGGCCGGCUGUCCUGGC	5	93	GCCAGGACAGCCGGCCCGA	472
93	CGCGAAAAGGUGGACAAGU	6	93	CGCGAAAAGGUGGACAAGU	6	111	ACUUGUCCACCUUUUCGCG	473
111	UCCUAUUUUCAAGAGAAGA	7	111	UCCUAUUUUCAAGAGAAGA	7	129	UCUUCUCUUGAAAAUAGGA	474
129	AUGACUUUUAACAGUUUUG	8	129	AUGACUUUUAACAGUUUUG	8	147	CAAAACUGUUAAAAGUCAU	475
147	GAAGGAUCUAAAACUUGUG	9	147	GAAGGAUCUAAAACUUGUG	9	165	CACAAGUUUUAGAUCCUUC	476
165	GUACCUGCAGACAUCAAUA	10	165	GUACCUGCAGACAUCAAUA	10	183	UAUUGAUGUCUGCAGGUAC	477
183	AAGGAAGAAGAAUUUGUAG	11	183	AAGGAAGAAGAAUUUGUAG	11	201	CUACAAAUUCUUCUUCCUU	478
201	GAAGAGUUUAAUAGAUUAA	12	201	GAAGAGUUUAAUAGAUUAA	12	219	UUAAUCUAUUAAACUCUUC	479
219	AAAACUUUUGCUAAUUUUC	13	219	AAAACUUUUGCUAAUUUUC	13	237	GAAAAUUAGCAAAAGUUUU	480
237	CCAAGUGGUAGUCCUGUUU	14	237	CCAAGUGGUAGUCCUGUUU	14	255	AAACAGGACUACCACUUGG	481
255	UCAGCAUCAACACUGGCAC	15	255	UCAGCAUCAACACUGGCAC	15	273	GUGCCAGUGUUGAUGCUGA	482
273	CGAGCAGGGUUUCUUUAUA	16	273	CGAGCAGGGUUUCUUUAUA	16	291	UAUAAAGAAACCCUGCUCG	483
291	ACUGGUGAAGGAGAUACCG	17	291	ACUGGUGAAGGAGAUACCG	17	309	CGGUAUCUCCUUCACCAGU	484
309	GUGCGGUGCUUUAGUUGUC	18	309	GUGCGGUGCUUUAGUUGUC	18	327	GACAACUAAAGCACCGCAC	485
327	CAUGCAGCUGUAGAUAGAU	19	327	CAUGCAGCUGUAGAUAGAU	19	345	AUCUAUCUACAGCUGCAUG	486
345	UGGCAAUAUGGAGACUCAG	20	345	UGGCAAUAUGGAGACUCAG	20	363	CUGAGUCUCCAUAUUGCCA	487
363	GCAGUUGGAAGACACAGGA	21	363	GCAGUUGGAAGACACAGGA	21	381	UCCUGUGUCUUCCAACUGC	488
381	AAAGUAUCCCCAAAUUGCA	22	381	AAAGUAUCCCCAAAUUGCA	22	399	UGCAAUUUGGGGAUACUUU	489
399	AGAUUUAUCAACGGCUUUU	23	399	AGAUUUAUCAACGGCUUUU	23	417	AAAAGCCGUUGAUAAAUCU	490
417	UAUCUUGAAAAUAGUGCCA	24	417	UAUCUUGAAAAUAGUGCCA	24	435	UGGCACUAUUUUCAAGAUA	491
435	ACGCAGUCUACAAAUUCUG	25	435	ACGCAGUCUACAAAUUCUG	25	453	CAGAAUUUGUAGACUGCGU	492
453	GGUAUCCAGAAUGGUCAGU	26	453	GGUAUCCAGAAUGGUCAGU	26	471	ACUGACCAUUCUGGAUACC	493
471	UACAAAGUUGAAAACUAUC	27	471	UACAAAGUUGAAAACUAUC	27	489	GAUAGUUUUCAACUUUGUA	494
489	CUGGGAAGCAGAGAUCAUU	28	489	CUGGGAAGCAGAGAUCAUU	28	507	AAUGAUCUCUGCUUCCCAG	495
507	UUUGCCUUAGACAGGCCAU	29	507	UUUGCCUUAGACAGGCCAU	29	525	AUGGCCUGUCUAAGGCAAA	496

525	UCUGAGACACAUGCAGACU	30	525	UCUGAGACACAUGCAGACU	30	543	AGUCUGCAUGUGUCUCAGA	497
543	UAUCUUUUGAGAACUGGGC	31	543	UAUCUUUUGAGAACUGGGC	31	561	GCCCAGUUCUCAAAAGAUA	498
561	CAGGUUGUAGAUAUAUCAG	32	561	CAGGUUGUAGAUAUAUCAG	32	579	CUGAUAUAUCUACAACCUG	499
579	GACACCAUAUACCCGAGGA	33	579	GACACCAUAUACCCGAGGA	33	597	UCCUCGGGUAUAUGGUGUC	500
597	AACCCUGCCAUGUAUAGUG	34	597	AACCCUGCCAUGUAUAGUG	34	615	CACUAUACAUGGCAGGGUU	501
615	GAAGAAGCUAGAUUAAAGU	35	615	GAAGAAGCUAGAUUAAAGU	35	633	ACUUUAAUCUAGCUUCUUC	502
633	UCCUUUCAGAACUGGCCAG	36	633	UCCUUUCAGAACUGGCCAG	36	651	CUGGCCAGUUCUGAAAGGA	503
651	GACUAUGCUCACCUAACCC	37	651	GACUAUGCUCACCUAACCC	37	669	GGGUUAGGUGAGCAUAGUC	504
669	CCAAGAGAGUUAGCAAGUG	38	669	CCAAGAGAGUUAGCAAGUG	38	687	CACUUGCUAACUCUCUUGG	505
687	GCUGGACUCUACUACACAG	39	687	GCUGGACUCUACUACACAG	39	705	CUGUGUAGUAGAGUCCAGC	506
705	GGUAUUGGUGACCAAGUGC	40	705	GGUAUUGGUGACCAAGUGC	40	723	GCACUUGGUCACCAAUACC	507
723	CAGUGCUUUUGUUGUGGUG	41	723	CAGUGCUUUUGUUGUGGUG	41	741	CACCACAACAAAAGCACUG	508
741	GGAAAACUGAAAAAUUGGG	42	741	GGAAAACUGAAAAAUUGGG	42	759	CCCAAUUUUUCAGUUUUCC	509
759	GAACCUUGUGAUCGUGCCU	43	759	GAACCUUGUGAUCGUGCCU	43	777	AGGCACGAUCACAAGGUUC	510
777	UGGUCAGAACACAGGCGAC	44	777	UGGUCAGAACACAGGCGAC	44	795	GUCGCCUGUGUUCUGACCA	511
795	CACUUUCCUAAUUGCUUCU	45	795	CACUUUCCUAAUUGCUUCU	45	813	AGAAGCAAUUAGGAAAGUG	512
813	UUUGUUUUGGGCCGGAAUC	46	813	UUUGUUUUGGGCCGGAAUC	46	831	GAUUCCGGCCCAAAACAAA	513
831	CUUAAUAUUCGAAGUGAAU	47	831	CUUAAUAUUCGAAGUGAAU	47	849	AUUCACUUCGAAUAUUAAG	514
849	UCUGAUGCUGUGAGUUCUG	48	849	UCUGAUGCUGUGAGUUCUG	48	867	CAGAACUCACAGCAUCAGA	515
867	GAUAGGAAUUUCCCAAAUU	49	867	GAUAGGAAUUUCCCAAAUU	49	885	AAUUUGGGAAAUUCCUAUC	516
885	UCAACAAAUCUUCCAAGAA	50	885	UCAACAAAUCUUCCAAGAA	50	903	UUCUUGGAAGAUUUGUUGA	517
903	AAUCCAUCCAUGGCAGAUU	51	903	AAUCCAUCCAUGGCAGAUU	51	921	AAUCUGCCAUGGAUGGAUU	518
921	UAUGAAGCACGGAUCUUUA	52	921	UAUGAAGCACGGAUCUUUA	52	939	UAAAGAUCCGUGCUUCAUA	519
939	ACUUUUGGGACAUGGAUAU	53	939	ACUUUUGGGACAUGGAUAU	53	957	AUAUCCAUGUCCCAAAAGU	520
957	UACUCAGUUAACAAGGAGC	54	957	UACUCAGUUAACAAGGAGC	54	975	GCUCCUUGUUAACUGAGUA	521
975	CAGCUUGCAAGAGCUGGAU	55	975	CAGCUUGCAAGAGCUGGAU	55	993	AUCCAGCUCUUGCAAGCUG	522
993	UUUUAUGCUUUAGGUGAAG	56	993	UUUUAUGCUUUAGGUGAAG	56	1011	CUUCACCUAAAGCAUAAAA	523
1011	GGUGAUAAAGUAAAGUGCU	57	1011	GGUGAUAAAGUAAAGUGCU	57	1029	AGCACUUUACUUUAUCACC	524
1029	UUUCACUGUGGAGGAGGGC	58	1029	UUUCACUGUGGAGGAGGGC	58	1047	GCCCUCCUCCACAGUGAAA	525
1047	CUAACUGAUUGGAAGCCCA	59	1047	CUAACUGAUUGGAAGCCCA	59	1065	UGGGCUUCCAAUCAGUUAG	526
1065	AGUGAAGACCCUUGGGAAC	60	1065	AGUGAAGACCCUUGGGAAC	60	1083	GUUCCCAAGGGUCUUCACU	527
1083	CAACAUGCUAAAUGGUAUC	61	1083	CAACAUGCUAAAUGGUAUC	61	1101	GAUACCAUUUAGCAUGUUG	528

1101	CCAGGGUGCAAAUAUCUGU	62	1101	CCAGGGUGCAAAUAUCUGU	62	1119	ACAGAUAUUUGCACCCUGG	529
1119	UUAGAACAGAAGGGACAAG	63	1119	UUAGAACAGAAGGGACAAG	63	1137	CUUGUCCCUUCUGUUCUAA	530
1137	GAAUAUAUAAACAAUAUUC	64	1137	GAAUAUAUAAACAAUAUUC	64	1155	GAAUAUUGUUUAUAUAUUC	531
1155	CAUUUAACUCAUUCACUUG	65	1155	CAUUUAACUCAUUCACUUG	65	1173	CAAGUGAAUGAGUUAAAUG	532
1173	GAGGAGUGUCUGGUAAGAA	66	1173	GAGGAGUGUCUGGUAAGAA	66	1191	UUCUUACCAGACACUCCUC	533
1191	ACUACUGAGAAAACACCAU	67	1191	ACUACUGAGAAAACACCAU	67	1209	AUGGUGUUUCUCAGUAGU	534
1209	UCACUAACUAGAAGAAUUG	68	1209	UCACUAACUAGAAGAAUUG	68	1227	CAAUUCUUCUAGUUAGUGA	535
1227	GAUGAUACCAUCUUCCAAA	69	1227	GAUGAUACCAUCUUCCAAA	69	1245	UUUGGAAGAUGGUAUCAUC	536
1245	AAUCCUAUGGUACAAGAAG	70	1245	AAUCCUAUGGUACAAGAAG	70	1263	CUUCUUGUACCAUAGGAUU	537
1263	GCUAUACGAAUGGGGUUCA	71	1263	GCUAUACGAAUGGGGUUCA	71	1281	UGAACCCCAUUCGUAUAGC	538
1281	AGUUUCAAGGACAUUAAGA	72	1281	AGUUUCAAGGACAUUAAGA	72	1299	UCUUAAUGUCCUUGAAACU	539
1299	AAAAUAAUGGAGGAAAAAA	73	1299	AAAAUAAUGGAGGAAAAAA	73	1317	UUUUUUCCUCCAUUAUUUU	540
1317	AUUCAGAUAUCUGGGAGCA	74	1317	AUUCAGAUAUCUGGGAGCA	74	1335	UGCUCCCAGAUAUCUGAAU	541
1335	AACUAUAAAUCACUUGAGG	75	1335	AACUAUAAAUCACUUGAGG	75	1353	CCUCAAGUGAUUUAUAGUU	542
1353	GUUCUGGUUGCAGAUCUAG	76	1353	GUUCUGGUUGCAGAUCUAG	76	1371	CUAGAUCUGCAACCAGAAC	543
1371	GUGAAUGCUCAGAAAGACA	77	1371	GUGAAUGCUCAGAAAGACA	77	1389	UGUCUUUCUGAGCAUUCAC	544
1389	AGUAUGCAAGAUGAGUCAA	78	1389	AGUAUGCAAGAUGAGUCAA	78	1407	UUGACUCAUCUUGCAUACU	545
1407	AGUCAGACUUCAUUACAGA	79	1407	AGUCAGACUUCAUUACAGA	79	1425	UCUGUAAUGAAGUCUGACU	546
1425	AAAGAGAUUAGUACUGAAG	80	1425	AAAGAGAUUAGUACUGAAG	80	1443	CUUCAGUACUAAUCUCUUU	547
1443	GAGCAGCUAAGGCGCCUGC	81	1443	GAGCAGCUAAGGCGCCUGC	81	1461	GCAGGCGCCUUAGCUGCUC	548
1461	CAAGAGGAGAAGCUUUGCA	82	1461	CAAGAGGAGAAGCUUUGCA	82	1479	UGCAAAGCUUCUCCUCUUG	549
1479	AAAAUCUGUAUGGAUAGAA	83	1479	AAAAUCUGUAUGGAUAGAA	83	1497	UUCUAUCCAUACAGAUUUU	550
1497	AAUAUUGCUAUCGUUUUUG	84	1497	AAUAUUGCUAUCGUUUUUG	84	1515	CAAAAACGAUAGCAAUAUU	551
1515	GUUCCUUGUGGACAUCUAG	85	1515	GUUCCUUGUGGACAUCUAG	85	1533	CUAGAUGUCCACACAAGGAAC	552
1533	GUCACUUGUAAACAAUGUG	86	1533	GUCACUUGUAAACAAUGUG	86	1551	CACAUUGUUUACAAGUGAC	553
1551	GCUGAAGCAGUUGACAAGU	87	1551	GCUGAAGCAGUUGACAAGU	87	1569	ACUUGUCAACUGCUUCAGC	554
1569	UGUCCCAUGUGCUACACAG	88	1569	UGUCCCAUGUGCUACACAG	88	1587	CUGUGUAGCACAUGGGACA	555
1587	GUCAUUACUUUUCAAGCAAA	89	1587	GUCAUUACUUUUCAAGCAAA	89	1605	UUUGCUUGAAAGUAAUGAC	556
1605	AAAAUUUUUAUGUCUUAAU	90	1605	AAAAUUUUUAUGUCUUAAU	90	1623	AUUAAGACAUAAAAAUUUU	557
1623	UCUAACUCUAUAGUAGGCA	91	1623	UCUAACUCUAUAGUAGGCA	91	1641	UGCCUACUAUAGAGUUAGA	558
1641	AUGUUAUGUUGUUCUUAUU	92	1641	AUGUUAUGUUGUUCUUAUU	92	1659	AAUAAGAACAACAUAAACAU	559
1659	UACCCUGAUUGAAUGUGUG	93	1659	UACCCUGAUUGAAUGUGUG	93	1677	CACACAUUCAAUCAGGGUA	560
1677	GAUGUGAACUGACUUUAAG	94	1677	GAUGUGAACUGACUUUAAG	94	1695	CUUAAAGUCAGUUCACAUC	561

1695	GUAAUCAGGAUUGAAUUCC	95	1695	GUAAUCAGGAUUGAAUUCC	95	1713	GGAAUUCAAUCCUGAUUAC	562
1713	CAUUAGCAUUUGCUACCAA	96	1713	CAUUAGCAUUUGCUACCAA	96	1731	UUGGUAGCAAAUGCUAAUG	563
1731	AGUAGGAAAAAAAAUGUAC	97	1731	AGUAGGAAAAAAAAUGUAC	97	1749	GUACAUUUUUUUUCCUACU	564
1749	CAUGGCAGUGUUUUAGUUG	98	1749	CAUGGCAGUGUUUUAGUUG	98	1767	CAACUAAAACACUGCCAUG	565
1767	GGCAAUAUAAUCUUUGAAU	99	1767	GGCAAUAUAAUCUUUGAAU	99	1785	AUUCAAAGAUUAUAUUGCC	566
1785	UUUCUUGAUUUUUUCAGGGU	100	1785	UUUCUUGAUUUUUUCAGGGU	100	1803	ACCCUGAAAAAUCAAGAAA	567
1803	UAUUAGCUGUAUUAUCCAU	101	1803	UAUUAGCUGUAUUAUCCAU	101	1821	AUGGAUAAUACAGCUAAUA	568
1821	UUUUUUUUACUGUUAUUUA	102	1821	UUUUUUUUACUGUUAUUUA	102	1839	UAAAUAACAGUAAAAAAAAA	569
1839	AAUUGAAACCAUAGACUAA	103	1839	AAUUGAAACCAUAGACUAA	103	1857	UUAGUCUAUGGUUUCAAUU	570
1857	AGAAUAAGAAGCAUCAUAC	104	1857	AGAAUAAGAAGCAUCAUAC	104	1875	GUAUGAUGCUUCUUAUUCU	571
1875	CUAUAACUGAACACAAUGU	105	1875	CUAUAACUGAACACAAUGU	105	1893	ACAUUGUGUUCAGUUAUAG	572
1893	UGUAUUCAUAGUAUACUGA	106	1893	UGUAUUCAUAGUAUACUGA	106	1911	UCAGUAUACUAUGAAUACA	573
1911	AUUUAAUUUCUAAGUGUAA	107	1911	AUUUAAUUUCUAAGUGUAA	107	1929	UUACACUUAGAAAUUAAAU	574
1929	AGUGAAUUAAUCAUCUGGA	108	1929	AGUGAAUUAAUCAUCUGGA	108	1947	UCCAGAUGAUUAAUUCACU	575
1947	AUUUUUUAUUCUUUUCAGA	109	1947	AUUUUUUAUUCUUUUCAGA	109	1965	UCUGAAAAGAAUAAAAAAU	576
1965	AUAGGCUUAACAAAUGGAG	110	1965	AUAGGCUUAACAAAUGGAG	110	1983	CUCCAUUUGUUAAGCCUAU	577
1983	GCUUUCUGUAUAUAAAUGU	111	1983	GCUUUCUGUAUAUAAAUGU	111	2001	ACAUUUAUAUACAGAAAGC	578
2001	UGGAGAUUAGAGUUAAUCU	112	2001	UGGAGAUUAGAGUUAAUCU	112	2019	AGAUUAACUCUAAUCUCCA	579
2019	UCCCCAAUCACAUAAUUUG	113	2019	UCCCCAAUCACAUAAUUUG	113	2037	CAAAUUAUGUGAUUGGGGA	580
2037	GUUUUGUGUGAAAAAGGAA	114	2037	GUUUUGUGUGAAAAAGGAA	114	2055	UUCCUUUUUCACACAAAAC	581
2055	AUAAAUUGUUCCAUGCUGG	115	2055	AUAAAUUGUUCCAUGCUGG	115	2073	CCAGCAUGGAACAAUUUAU	582
2073	GUGGAAAGAUAGAGAUUGU	116	2073	GUGGAAAGAUAGAGAUUGU	116	2091	ACAAUCUCUAUCUUUCCAC	583
2091	UUUUUAGAGGUUGGUUGUU	117	2091	UUUUUAGAGGUUGGUUGUU	117	2109	AACAACCAACCUCUAAAAA	584
2109	UGUGUUUUAGGAUUCUGUC	118	2109	UGUGUUUUAGGAUUCUGUC	118	2127	GACAGAAUCCCUAAAACACA	585
2127	CCAUUUUCUUUUAAAGUUA	119	2127	CCAUUUUCUUUUAAAGUUA	119	2145	UAACUUUAAAAGAAAAUGG	586
2145	AUAAACACGUACUUGUGCG	120	2145	AUAAACACGUACUUGUGCG	120	2163	CGCACAAGUACGUGUUUAU	587
2163	GAAUUAUUUUUUUAAAGUG	121	2163	GAAUUAUUUUUUUAAAGUG	121	2181	CACUUUAAAAAAAUAAUUC	588
2181	GAUUUGCCAUUUUUGAAAG	122	2181	GAUUUGCCAUUUUUGAAAG	122	2199	CUUUCAAAAAUGGCAAAUC	589
2199	GCGUAUUUAAUGAUAGAAU	123	2199	GCGUAUUUAAUGAUAGAAU	123	2217	AUUCUAUCAUUAAAAUACGC	590
2217	UACUAUCGAGCCAACAUGU	124	2217	UACUAUCGAGCCAACAUGU	124	2235	ACAUGUUGGCUCGAUAGUA	591
2235	UACUGACAUGGAAAGAUGU	125	2235	UACUGACAUGGAAAGAUGU	125	2253	ACAUCUUUCCAUGUCAGUA	592
2253	UCAAAGAUAUGUUAAGUGU	126	2253	UCAAAGAUAUGUUAAGUGU	126	2271	ACACUUAACAUAUCUUUGA	593

2271	UAAAAUGCAAGUGGCAAAA	127	2271	UAAAAUGCAAGUGGCAAAA	127	2289	UUUUGCCACUUGCAUUUUA	594
2289	ACACUAUGUAUAGUCUGAG	128	2289	ACACUAUGUAUAGUCUGAG	128	2307	CUCAGACUAUACAUAGUGU	595
2307	GCCAGAUCAAAGUAUGUAU	129	2307	GCCAGAUCAAAGUAUGUAU	129	2325	AUACAUACUUUGAUCUGGC	596
2325	UGUUUUUAAUAUGCAUAGA	130	2325	UGUUUUUAAUAUGCAUAGA	130	2343	UCUAUGCAUAUUAAAAACA	597
2343	AACAAAAGAUUUGGAAAGA	131	2343	AACAAAAGAUUUGGAAAGA	131	2361	UCUUUCCAAAUCUUUUGUU	598
2361	AUAUACACCAAACUGUUAA	132	2361	AUAUACACCAAACUGUUAA	132	2379	UUAACAGUUUGGUGUAUAU	599
2379	AAUGUGGUUUCUCUUCGGG	133	2379	AAUGUGGUUUCUCUUCGGG	133	2397	CCCGAAGAGAAACCACAUU	600
2397	GGAGGGGGGGAUUGGGGGA	134	2397	GGAGGGGGGGAUUGGGGGA	134	2415	UCCCCCAAUCCCCCCCUCC	601
2415	AGGGGCCCCAGAGGGGUUU	135	2415	AGGGGCCCCAGAGGGGUUU	135	2433	AAACCCCUCUGGGGCCCCU	602
2433	UUAUAGGGGCCUUUUCACU	136	2433	UUAUAGGGGCCUUUUCACU	136	2451	AGUGAAAAGGCCCCUAUAA	603
2451	UUUCUACUUUUUUCAUUUU	137	2451	UUUCUACUUUUUUCAUUUU	137	2469	AAAAUGAAAAAAGUAGAAA	604
2469	UGUUCUGUUCGAAUUUUUU	138	2469	UGUUCUGUUCGAAUUUUUU	138	2487	AAAAAAUUCGAACAGAACA	605
2487	UAUAAGUAUGUAUUACUUU	139	2487	UAUAAGUAUGUAUUACUUU	139	2505	AAAGUAAUACAUACUUAUA	606
2505	UUGUAAUCAGAAUUUUUAG	140	2505	UUGUAAUCAGAAUUUUUAG	140	2523	CUAAAAAUUCUGAUUACAA	607
2523	GAAAGUAUUUUGCUGAUUU	141	2523	GAAAGUAUUUUGCUGAUUU	141	2541	AAAUCAGCAAAAUACUUUC	608
2541	UAAAGGCUUAGGCAUGUUC	142	2541	UAAAGGCUUAGGCAUGUUC	142	2559	GAACAUGCCUAAGCCUUUA	609
2559	CAAACGCCUGCAAAACUAC	143	2559	CAAACGCCUGCAAAACUAC	143	2577	GUAGUUUUGCAGGCGUUUG	610
2577	CUUAUCACUCAGCUUUAGU	144	2577	CUUAUCACUCAGCUUUAGU	144	2595	ACUAAAGCUGAGUGAUAAG	611
2595	UUUUUCUAAUCCAAGAAGG	145	2595	UUUUUCUAAUCCAAGAAGG	145	2613	CCUUCUUGGAUUAGAAAAA	612
2613	GCAGGGCAGUUAACCUUUU	146	2613	GCAGGGCAGUUAACCUUUU	146	2631	AAAAGGUUAACUGCCCUGC	613
2631	UUGGUGCCAAUGUGAAAUG	147	2631	UUGGUGCCAAUGUGAAAUG	147	2649	CAUUUCACAUUGGCACCAA	614
2649	GUAAAUGAUUUUAUGUUUU	148	2649	GUAAAUGAUUUUAUGUUUU	148	2667	AAAACAUAAAAUCAUUUAC	615
2667	UUCCUGCUUUGUGGAUGAA	149	2667	UUCCUGCUUUGUGGAUGAA	149	2685	UUCAUCCACAAAGCAGGAA	616
2685	AAAAUAUUUCUGAGUGGUA	150	2685	AAAAUAUUUCUGAGUGGUA	150	2703	UACCACUCAGAAAUAUUUU	617
2703	AGUUUUUUGACAGGUAGAC	151	2703	AGUUUUUUGACAGGUAGAC	151	2721	GUCUACCUGUCAAAAAACU	618
2721	CCAUGUCUUAUCUUGUUUC	152	2721	CCAUGUCUUAUCUUGUUUC	152	2739	GAAACAAGAUAAGACAUGG	619
2739	CAAAAUAAGUAUUUCUGAU	153	2739	CAAAAUAAGUAUUUCUGAU	153	2757	AUCAGAAAUACUUAUUUUG	620
2757	UUUUGUAAAAUGAAAUAUA	154	2757	UUUUGUAAAAUGAAAUAUA	154	2775	UAUAUUUCAUUUUACAAAA	621
2775	AAAAUAUGUCUCAGAUCUU	155	2775	AAAAUAUGUCUCAGAUCUU	155	2793	AAGAUCUGAGACAUAUUUU	622
2793	UCCAAUUAAUUAGUAAGGA	156	2793	UCCAAUUAAUUAGUAAGGA	156	2811	UCCUUACUAAUUAAUUGGA	623
2811	AUUCAUCCUUAAUCCUUGC	157	2811	AUUCAUCCUUAAUCCUUGC	157	2829	GCAAGGAUUAAGGAUGAAU	624
2829	CUAGUUUAAGCCUGCCUAA	158	2829	CUAGUUUAAGCCUGCCUAA	158	2847	UUAGGCAGGCUUAAACUAG	625
2847	AGUCACUUUACUAAAAGAU	159	2847	AGUCACUUUACUAAAAGAU	159	2865	AUCUUUUAGUAAAGUGACU	626

2865	UCUUUGUUAACUCAGUAUU	160	2865	UCUUUGUUAACUCAGUAUU	160	2883	AAUACUGAGUUAACAAAGA	627
2883	UUUAAACAUCUGUCAGCUU	161	2883	UUUAAACAUCUGUCAGCUU	161	2901	AAGCUGACAGAUGUUUAAA	628
2901	UAUGUAGGUAAAAGUAGAA	162	2901	UAUGUAGGUAAAAGUAGAA	162	2919	UUCUACUUUUACCUACAUA	629
2919	AGCAUGUUUGUACACUGCU	163	2919	AGCAUGUUUGUACACUGCU	163	2937	AGCAGUGUACAAACAUGCU	630
2937	UUGUAGUUAUAGUGACAGC	164	2937	UUGUAGUUAUAGUGACAGC	164	2955	GCUGUCACUAUAACUACAA	631
2955	CUUUCCAUGUUGAGAUUCU	165	2955	CUUUCCAUGUUGAGAUUCU	165	2973	AGAAUCUCAACAUGGAAAG	632
2973	UCAUAUCAUCUUGUAUCUU	166	2973	UCAUAUCAUCUUGUAUCUU	166	2991	AAGAUACAAGAUGAUAUGA	633
2991	UAAAGUUUCAUGUGAGUUU	167	2991	UAAAGUUUCAUGUGAGUUU	167	3009	AAACUCACAUGAAACUUUA	634
3009	UUUACCGUUAGGAUGAUUA	168	3009	UUUACCGUUAGGAUGAUUA	168	3027	UAAUCAUCCUAACGGUAAA	635
3027	AAGAUGUAUAUAGGACAAA	169	3027	AAGAUGUAUAUAGGACAAA	169	3045	UUUGUCCUAUAUACAUCUU	636
3045	AAUGUUAAGUCUUUCCUCU	170	3045	AAUGUUAAGUCUUUCCUCU	170	3063	AGAGGAAAGACUUAACAUU	637
3063	UACCUACAUUUGUUUUCUU	171	3063	UACCUACAUUUGUUUUCUU	171	3081	AAGAAAACAAAUGUAGGUA	638
3081	UGGCUAGUAAUAGUAGUAG	172	3081	UGGCUAGUAAUAGUAGUAG	172	3099	CUACUACUAUUACUAGCCA	639
3099	GAUACUUCUGAAAUAAAUG	173	3099	GAUACUUCUGAAAUAAAUG	173	3117	CAUUUAUUUCAGAAGUAUC	640
3117	GUUCUCUCAAGAUCCUUAA	174	3117	GUUCUCUCAAGAUCCUUAA	174	3135	UUAAGGAUCUUGAGAGAAC	641
3135	AAACCUCUUGGAAAUUAUA	175	3135	AAACCUCUUGGAAAUUAUA	175	3153	UAUAAUUUCCAAGAGGUUU	642
3153	AAAAAUAUUGGCAAGAAAA	176	3153	AAAAAUAUUGGCAAGAAAA	176	3171	UUUUCUUGCCAAUAUUUUU	643
3171	AGAAGAAUAGUUGUUUAAA	177	3171	AGAAGAAUAGUUGUUUAAA	177	3189	UUUAAACAACUAUUCUUCU	644
3189	AUAUUUUUUAAAAAACACU	178	3189	AUAUUUUUUAAAAAACACU	178	3207	AGUGUUUUUUAAAAAAUAU	645
3207	UUGAAUAAGAAUCAGUAGG	179	3207	UUGAAUAAGAAUCAGUAGG	179	3225	CCUACUGAUUCUUAUUCAA	646
3225	GGUAUAAACUAGAAGUUUA	180	3225	GGUAUAAACUAGAAGUUUA	180	3243	UAAACUUCUAGUUUAUACC	647
3243	AAAAAUGCUUCAUAGAACG	181	3243	AAAAAUGCUUCAUAGAACG	181	3261	CGUUCUAUGAAGCAUUUUU	648
3261	GUCCAGGGUUUACAUUACA	182	3261	GUCCAGGGUUUACAUUACA	182	3279	UGUAAUGUAAACCCUGGAC	649
3279	AAGAUUCUCACAACAAACC	183	3279	AAGAUUCUCACAACAAACC	183	3297	GGUUUGUUGUGAGAAUCUU	650
3297	CUAUUGUAGAGGUGAGUAA	184	3297	CUAUUGUAGAGGUGAGUAA	184	3315	UUACUCACCUCUACAAUAG	651
3315	AGGCAUGUUACUACAGAGG	185	3315	AGGCAUGUUACUACAGAGG	185	3333	CCUCUGUAGUAACAUGCCU	652
3333	GAAAGUUUGAGAGUAAAAC	186	3333	GAAAGUUUGAGAGUAAAAC	186	3351	GUUUUACUCUCAAACUUUC	653
3351	CUGUAAAAAAUUAUAUUUU	187	3351	CUGUAAAAAAUUAUAUUUU	187	3369	AAAAUAUAAUUUUUUACAG	654
3369	UUGUUGUACUUUCUAAGAG	188	3369	UUGUUGUACUUUCUAAGAG	188	3387	CUCUUAGAAAGUACAACAA	655
3387	GAAAGAGUAUUGUUAUGUU	189	3387	GAAAGAGUAUUGUUAUGUU	189	3405	AACAUAACAAUACUCUUUC	656
3405	UCUCCUAACUUCUGUUGAU	190	3405	UCUCCUAACUUCUGUUGAU	190	3423	AUCAACAGAAGUUAGGAGA	657
3423	UUACUACUUUAAGUGAUAU	191	3423	UUACUACUUUAAGUGAUAU	191	3441	AUAUCACUUAAAGUAGUAA	658
3441	UUCAUUUAAAACAUUGCAA	192	3441	UUCAUUUAAAACAUUGCAA	192	3459	UUGCAAUGUUUUAAAUGAA	659

3459	AAUUUAUUUUAUUUAUUUA	193	3459	AAUUUAUUUUAUUUAUUUA	193	3477	UAAAUAAAUAAAAUAAAUU	660
3477	AAUUUUCUUUUUGAGAUGG	194	3477	AAUUUUCUUUUUGAGAUGG	194	3495	CCAUCUCAAAAAGAAAAUU	661
3495	GAGUCUUGCUUGUCACCCA	195	3495	GAGUCUUGCUUGUCACCCA	195	3513	UGGGUGACAAGCAAGACUC	662
3513	AGGCUGGAGUGCAGUGGAG	196	3513	AGGCUGGAGUGCAGUGGAG	196	3531	CUCCACUGCACUCCAGCCU	663
3531	GUGAUCUCUGCUCACUGCA	197	3531	GUGAUCUCUGCUCACUGCA	197	3549	UGCAGUGAGCAGAGAUCAC	664
3549	AACCUCCGCCUUCUGGGUU	198	3549	AACCUCCGCCUUCUGGGUU	198	3567	AACCCAGAAGGCGGAGGUU	665
3567	UCAAGCGAUUCUCGUGCCU	199	3567	UCAAGCGAUUCUCGUGCCU	199	3585	AGGCACGAGAAUCGCUUGA	666
3585	UCAGCUUCCUGAGUAGCUG	200	3585	UCAGCUUCCUGAGUAGCUG	200	3603	CAGCUACUCAGGAAGCUGA	667
3603	GGAAUUACAGGCAGGUGCC	201	3603	GGAAUUACAGGCAGGUGCC	201	3621	GGCACCUGCCUGUAAUUCC	668
3621	CACCAUGCCCGACUAAUUU	202	3621	CACCAUGCCCGACUAAUUU	202	3639	AAAUUAGUCGGGCAUGGUG	669
3639	UUUUUUUAUUUUUAGUAGA	203	3639	UUUUUUUAUUUUUAGUAGA	203	3657	UCUACUAAAAAUAAAAAAA	670
3657	AGACGGGGUUUCACCAUGU	204	3657	AGACGGGGUUUCACCAUGU	204	3675	ACAUGGUGAAACCCCGUCU	671
3675	UUGGCCAGGCUGGUAUCAA	205	3675	UUGGCCAGGCUGGUAUCAA	205	3693	UUGAUACCAGCCUGGCCAA	672
3693	AACUCCUGACCUCAAGAGA	206	3693	AACUCCUGACCUCAAGAGA	206	3711	UCUCUUGAGGUCAGGAGUU	673
3711	AUCCACUCGCCUUGCCCUC	207	3711	AUCCACUCGCCUUGCCCUC	207	3729	GAGGGCAAGGCGAGUGGAU	674
3729	CCCAAAGUGCUGGGAUUAC	208	3729	CCCAAAGUGCUGGGAUUAC	208	3747	GUAAUCCCAGCACUUUGGG	675
3747	CAGGCUUGAGCCACCACGC	209	3747	CAGGCUUGAGCCACCACGC	209	3765	GCGUGGUGGCUCAAGCCUG	676
3765	CCCGGCUAAAACAUUGCAA	210	3765	CCCGGCUAAAACAUUGCAA	210	3783	UUGCAAUGUUUUAGCCGGG	677
3783	AAUUUAAAUGAGAGUUUUA	211	3783	AAUUUAAAUGAGAGUUUUA	211	3801	UAAAACUCUCAUUUAAAUU	678
3801	AAAAAUUAAAUAAUGACUG	212	3801	AAAAAUUAAAUAAUGACUG	212	3819	CAGUCAUUAUUUAAUUUUU	679
3819	GCCCUGUUUCUGUUUUAGU	213	3819	GCCCUGUUUCUGUUUUAGU	213	3837	ACUAAAACAGAAACAGGGC	680
3837	UAUGUAAAUCCUCAGUUCU	214	3837	UAUGUAAAUCCUCAGUUCU	214	3855	AGAACUGAGGAUUUACAUA	681
3855	UUCACCUUUGCACUGUCUG	215	3855	UUCACCUUUGCACUGUCUG	215	3873	CAGACAGUGCAAAGGUGAA	682
3873	GCCACUUAGUUUGGUUAUA	216	3873	GCCACUUAGUUUGGUUAUA	216	3891	UAUAACCAAACUAAGUGGC	683
3891	AUAGUCAUUAACUUGAAUU	217	3891	AUAGUCAUUAACUUGAAUU	217	3909	AAUUCAAGUUAAUGACUAU	684
3909	UUGGUCUGUAUAGUCUAGA	218	3909	UUGGUCUGUAUAGUCUAGA	218	3927	UCUAGACUAUACAGACCAA	685
3927	ACUUUAAAUUUAAAGUUUU	219	3927	ACUUUAAAUUUAAAGUUUU	219	3945	AAAACUUUAAAUUUAAAGU	686
3945	UCUACAAGGGAGAAAAGU	220	3945	UCUACAAGGGGAGAAAAGU	220	3963	ACUUUUCUCCCCCUUGUAGA	687
3963	UGUUAAAAUUUUUAAAAUA	221	3963	UGUUAAAAUUUUUAAAAUA	221	3981	UAUUUUAAAAAUUUUAACA	688
3981	AUGUUUUCCAGGACACUUC	222	3981	AUGUUUUCCAGGACACUUC	222	3999	GAAGUGUCCUGGAAAACAU	689
3999	CACUUCCAAGUCAGGUAGG	223	3999	CACUUCCAAGUCAGGUAGG	223	4017	CCUACCUGACUUGGAAGUG	690
4017	GUAGUUCAAUCUAGUUGUU	224	4017	GUAGUUCAAUCUAGUUGUU	224	4035	AACAACUAGAUUGAACUAC	691
4035	UAGCCAAGGACUCAAGGAC	225	4035	UAGCCAAGGACUCAAGGAC	225	4053	GUCCUUGAGUCCUUGGCUA	692

4053	CUGAAUUGUUUUAACAUAA	226	4053	CUGAAUUGUUUUAACAUAA	226	4071	UUAUGUUAAAACAAUUCAG	693
4071	AGGCUUUUCCUGUUCUGGG	227	4071	AGGCUUUUCCUGUUCUGGG	227	4089	CCCAGAACAGGAAAAGCCU	694
4089	GAGCCGCACUUCAUUAAAA	228	4089	GAGCCGCACUUCAUUAAAA	228	4107	UUUUAAUGAAGUGCGGCUC	695
4107	AUUCUUCUAAAACUUGUAU	229	4107	AUUCUUCUAAAACUUGUAU	229	4125	AUACAAGUUUUAGAAGAAU	696
4125	UGUUUAGAGUUAAGCAAGA	230	4125	UGUUUAGAGUUAAGCAAGA	230	4143	UCUUGCUUAACUCUAAACA	697
4143	ACUUUUUUUCUUCCUCUCC	231	4143	ACUUUUUUUCUUCCUCUCC	231	4161	GGAGAGGAAGAAAAAAAGU	698
4161	CAUGAGUUGUGAAAUUUAA	232	4161	CAUGAGUUGUGAAAUUUAA	232	4179	UUAAAUUUCACAACUCAUG	699
4179	AUGCACAACGCUGAUGUGG	233	4179	AUGCACAACGCUGAUGUGG	233	4197	CCACAUCAGCGUUGUGCAU	700
4197	GCUAACAAGUUUAUUUUAA	234	4197	GCUAACAAGUUUAUUUUAA	234	4215	UUAAAAUAAACUUGUUAGC	701
4215	AGAAUUGUUUAGAAAUGCU	235	4215	AGAAUUGUUUAGAAAUGCU	235	4233	AGCAUUUCUAAACAAUUCU	702
4233	UGUUGCUUCAGGUUCUUAA	236	4233	UGUUGCUUCAGGUUCUUAA	236	4251	UUAAGAACCUGAAGCAACA	703
4251	AAAUCACUCAGCACUCCAA	237	4251	AAAUCACUCAGCACUCCAA	237	4269	UUGGAGUGCUGAGUGAUUU	704
4269	ACUUCUAAUCAAAUUUUUG	238	4269	ACUUCUAAUCAAAUUUUUG	238	4287	CAAAAAUUUGAUUAGAAGU	705
4287	GGAGACUUAACAGCAUUUG	239	4287	GGAGACUUAACAGCAUUUG	239	4305	CAAAUGCUGUUAAGUCUCC	706
4305	GUCUGUGUUUGAACUAUAA	240	4305	GUCUGUGUUUGAACUAUAA	240	4323	UUAUAGUUCAAACACAGAC	707
4323	AAAAGCACCGGAUCUUUUC	241	4323	AAAAGCACCGGAUCUUUUC	241	4341	GAAAAGAUCCGGUGCUUUU	708
4341	CCAUCUAAUUCCGCAAAAA	242	4341	CCAUCUAAUUCCGCAAAAA	242	4359	UUUUUGCGGAAUUAGAUGG	709
4359	AUUGAUCAUUUGCAAAGUC	243	4359	AUUGAUCAUUUGCAAAGUC	243	4377	GACUUUGCAAAUGAUCAAU	710
4377	CAAAACUAUAGCCAUAUCC	244	4377	CAAAACUAUAGCCAUAUCC	244	4395	GGAUAUGGCUAUAGUUUUG	711
4395	CAAAUCUUUUCCCCCUCCC	245	4395	CAAAUCUUUUCCCCCUCCC	245	4413	GGGAGGGGGAAAAGAUUUG	712
4413	CAAGAGUUCUCAGUGUCUA	246	4413	CAAGAGUUCUCAGUGUCUA	246	4431	UAGACACUGAGAACUCUUG	713
4431	ACAUGUAGACUAUUCCUUU	247	4431	ACAUGUAGACUAUUCCUUU	247	4449	AAAGGAAUAGUCUACAUGU	714
4449	UUCUGUAUAAAGUUCACUC	248	4449	UUCUGUAUAAAGUUCACUC	248	4467	GAGUGAACUUUAUACAGAA	715
4467	CUAGGAUUUCAAGUCACCA	249	4467	CUAGGAUUUCAAGUCACCA	249	4485	UGGUGACUUGAAAUCCUAG	716
4485	ACUUAUUUUACAUUUUAGU	250	4485	ACUUAUUUUACAUUUUAGU	250	4503	ACUAAAAUGUAAAAUAAGU	717
4503	UCAUGCAAAGAUUCAAGUA	251	4503	UCAUGCAAAGAUUCAAGUA	251	4521	UACUUGAAUCUUUGCAUGA	718
4521	AGUUUUGCAAUAAGUACUU	252	4521	AGUUUUGCAAUAAGUACUU	252	4539	AAGUACUUAUUGCAAAACU	719
4539	UAUCUUUAUUUGUAAUAAU	253	4539	UAUCUUUAUUUGUAAUAAU	253	4557	AUUAUUACAAAUAAAGAUA	720
4557	UUUAGUCUGCUGAUCAAAA	254	4557	UUUAGUCUGCUGAUCAAAA	254	4575	UUUUGAUCAGCAGACUAAA	721
4575	AGCAUUGUCUUAAUUUUUG	255	4575	AGCAUUGUCUUAAUUUUUG	255	4593	CAAAAAUUAAGACAAUGCU	722
4593	GAGAACUGGUUUUAGCAUU	256	4593	GAGAACUGGUUUUAGCAUU	256	4611	AAUGCUAAAAACCAGUUCUC	723
4611	UUACAAACUAAAUUCCAGU	257	4611	UUACAAACUAAAUUCCAGU	257	4629	ACUGGAAUUUAGUUUGUAA	724
4629	UUAAUUAAUUAAUAGCUUU	258	4629	UUAAUUAAUUAAUAGCUUU	258	4647	AAAGCUAUUAAUUAAUUAA	725

4647	UAUAUUGCCUUUCCUGCUA	259	4647	UAUAUUGCCUUUCCUGCUA	259	4665	UAGCAGGAAAGGCAAUAUA	726
4665	ACAUUUGGUUUUUUCCCCU	260	4665	ACAUUUGGUUUUUUCCCCU	260	4683	AGGGGAAAAAACCAAAUGU	727
4683	UGUCCCUUUGAUUACGGGC	261	4683	UGUCCCUUUGAUUACGGGC	261	4701	GCCCGUAAUCAAAGGGACA	728
4701	CUAAGGUAGGGUAGAGUGG	262	4701	CUAAGGUAGGGUAGAGUGG	262	4719	CCACUCUACCCUACCUUAG	729
4719	GGUGUAGUGAGUGUAUAUA	263	4719	GGUGUAGUGAGUGUAUAUA	263	4737	UAUAUACACUCACUACACC	730
4737	AAUGUGAUUUGGCCCUGUG	264	4737	AAUGUGAUUUGGCCCUGUG	264	4755	CACAGGGCCAAAUCACAUU	731
4755	GUAUUAUGAUAUUUUGUUA	265	4755	GUAUUAUGAUAUUUUGUUA	265	4773	UAACAAAAUAUCAUAAUAC	732
4773	AUUUUUGUUGUUAUAUUAU	266	4773	AUUUUUGUUGUUAUAUUAU	266	4791	AUAAUAUAACAACAAAAAU	733
4791	UUUACAUUUCAGUAGUUGU	267	4791	UUUACAUUUCAGUAGUUGU	267	4809	ACAACUACUGAAAUGUAAA	734
4809	UUUUUUGUGUUUCCAUUUU	268	4809	UUUUUUGUGUUUCCAUUUU	268	4827	AAAAUGGAAACACAAAAAA	735
4827	UAGUGGAUAAAAUUUGUAU	269	4827	UAGUGGAUAAAAUUUGUAU	269	4845	AUACAAAUUUUAUCCACUA	736
4845	UUUUGAACUAUGAAUGGAG	270	4845	UUUUGAACUAUGAAUGGAG	270	4863	CUCCAUUCAUAGUUCAAAA	737
4863	GACUACCGCCCCAGCAUUA	271	4863	GACUACCGCCCCAGCAUUA	271	4881	UAAUGCUGGGGCGGUAGUC	738
4881	AGUUUCACAUGAUAUACCC	272	4881	AGUUUCACAUGAUAUACCC	272	4899	GGGUAUAUCAUGUGAAACU	739
4899	CUUUAAACCCGAAUCAUUG	273	4899	CUUUAAACCCGAAUCAUUG	273	4917	CAAUGAUUCGGGUUUAAAG	740
4917	GUUUUAUUUCCUGAUUACA	274	4917	GUUUUAUUUCCUGAUUACA	274	4935	UGUAAUCAGGAAAUAAAAC	741
4935	ACAGGUGUUGAAUGGGGAA	275	4935	ACAGGUGUUGAAUGGGGAA	275	4953	UUCCCCAUUCAACACCUGU	742
4953	AAGGGGCUAGUAUAUCAGU	276	4953	AAGGGGCUAGUAUAUCAGU	276	4971	ACUGAUAUACUAGCCCCUU	743
4971	UAGGAUAUACUAUGGGAUG	277	4971	UAGGAUAUACUAUGGGAUG	277	4989	CAUCCCAUAGUAUAUCCUA	744
4989	GUAUAUAUAUCAUUGCUGU	278	4989	GUAUAUAUAUCAUUGCUGU	278	5007	ACAGCAAUGAUAUAUAUAC	745
5007	UUAGAGAAAUGAAAUAAAA	279	5007	UUAGAGAAAUGAAAUAAAA	279	5025	UUUUAUUUCAUUUCUCUAA	746
5025	AUGGGGCUGGGCUCAGUGG	280	5025	AUGGGGCUGGGCUCAGUGG	280	5043	CCACUGAGCCCAGCCCCAU	747
5043	GCUCACGCCUGUAAUCCCA	281	5043	GCUCACGCCUGUAAUCCCA	281	5061	UGGGAUUACAGGCGUGAGC	748
5061	AGCACUUUGGGAGGCUGAG	282	5061	AGCACUUUGGGAGGCUGAG	282	5079	CUCAGCCUCCCAAAGUGCU	749
5079	GGCAGGUGGAUCACGAGGU	283	5079	GGCAGGUGGAUCACGAGGU	283	5097	ACCUCGUGAUCCACCUGCC	750
5097	UCAGGAGAUCGAGACCAUC	284	5097	UCAGGAGAUCGAGACCAUC	284	5115	GAUGGUCUCGAUCUCCUGA	751
5115	CCUGGCUAACACGGUGAAA	285	5115	CCUGGCUAACACGGUGAAA	285	5133	UUUCACCGUGUUAGCCAGG	752
5133	ACCCCGUCUCUACUAAAAA	286	5133	ACCCCGUCUCUACUAAAAA	286	5151	UUUUUAGUAGAGACGGGGU	753
5151	AACAGAAAAUUAGCCGGGC	287	5151	AACAGAAAAUUAGCCGGGC	287	5169	GCCCGGCUAAUUUUCUGUU	754
5169	CGUGGUGGCGGGCGCCUGU	288	5169	CGUGGUGGCGGGCGCCUGU	288	5187	ACAGGCGCCCGCCACCACG	755
5187	UAGUCCCAGCUACUCGGGA	289	5187	UAGUCCCAGCUACUCGGGA	289	5205	UCCCGAGUAGCUGGGACUA	756
5205	AGGCUGAGGCAGGAGAAUG	290	5205	AGGCUGAGGCAGGAGAAUG	290	5223	CAUUCUCCUGCCUCAGCCU	757
5223	GGUGUGAACCCGGGAGGCA	291	5223	GGUGUGAACCCGGGAGGCA	291	5241	UGCCUCCCGGGUUCACACC	758

5241	AGAGCUUGCAGUGAGCCGA	292	5241	AGAGCUUGCAGUGAGCCGA	292	5259	UCGGCUCACUGCAAGCUCU	759
5259	AGAUCUCGCCACUGCACUC	293	5259	AGAUCUCGCCACUGCACUC	293	5277	GAGUGCAGUGGCGAGAUCU	760
5277	CCAGCCUGGGCAACAGAGC	294	5277	CCAGCCUGGGCAACAGAGC	294	5295	GCUCUGUUGCCCAGGCUGG	761
5295	CAAGACUCUGUCUCAAAAA	295	5295	CAAGACUCUGUCUCAAAAA	295	5313	UUUUUGAGACAGAGUCUUG	762
5313	AAAAAAAAAAAAGAAAUAA	296	5313	AAAAAAAAAAAAGAAAUAA	296	5331	UUAUUUCUUUUUUUUUUUU	763
5331	AGAAAAUGGGAAGCAAUAU	297	5331	AGAAAAUGGGAAGCAAUAU	297	5349	AUAUUGCUUCCCAUUUUCU	764
5349	UUUGACAUAGUUCUUUUUA	298	5349	UUUGACAUAGUUCUUUUUA	298	5367	UAAAAAGAACUAUGUCAAA	765
5367	AGUCAAAUCUACUUGUUAA	299	5367	AGUCAAAUCUACUUGUUAA	299	5385	UUAACAAGUAGAUUUGACU	766
5385	AAAAAAGGGUAGCAGUUUA	300	5385	AAAAAAGGGUAGCAGUUUA	300	5403	UAAACUGCUACCCUUUUUU	767
5403	AUUCAUCUGUGAAAGGAAA	301	5403	AUUCAUCUGUGAAAGGAAA	301	5421	UUUCCUUUCACAGAUGAAU	768
5421	AAUAAUACUUAUCUUACAA	302	5421	AAUAAUACUUAUCUUACAA	302	5439	UUGUAAGAUAAGUAUUAUU	769
5439	AGGUUGCAAGAGCUCAAGG	303	5439	AGGUUGCAAGAGCUCAAGG	303	5457	CCUUGAGCUCUUGCAACCU	770
5457	GAGACCAUGUAUGUAAAGU	304	5457	GAGACCAUGUAUGUAAAGU	304	5475	ACUUUACAUACAUGGUCUC	771
5475	UUCCUGCUGUAAAUAUGAA	305	5475	UUCCUGCUGUAAAUAUGAA	305	5493	UUCAUAUUUACAGCAGGAA	772
5493	ACUCCCAUCCUAAUACCCU	306	5493	ACUCCCAUCCUAAUACCCU	306	5511	AGGGUAUUAGGAUGGGAGU	773
5511	UUUUACCUCUCUGUGGGUU	307	5511	UUUUACCUCUCUGUGGGUU	307	5529	AACCCACAGAGAGGUAAAA	774
5529	UUGUCUUGACCUGGAAAUU	308	5529	UUGUCUUGACCUGGAAAUU	308	5547	AAUUUCCAGGUCAAGACAA	775
5547	UUGGGCUAAAACUUAGAAA	309	5547	UUGGGCUAAAACUUAGAAA	309	5565	UUUCUAAGUUUUAGCCCAA	776
5565	AAAAUUCUUACAUGAUAAC	310	5565	AAAAUUCUUACAUGAUAAC	310	5583	GUUAUCAUGUAAGAAUUUU	777
5583	CUCAGUGAUGCUUACUCAU	311	5583	CUCAGUGAUGCUUACUCAU	311	5601	AUGAGUAAGCAUCACUGAG	778
5601	UAGUUUUUGGUGUUUCUCA	312	5601	UAGUUUUUGGUGUUUCUCA	312	5619	UGAGAAACACCAAAAACUA	779
5619	AUAGAUAAGAUAUAAAUCA	313	5619	AUAGAUAAGAUAUAAAUCA	313	5637	UGAUUUAUAUCUUAUCUAU	780
5637	AGCUGGGCGCGGUGGCUCA	314	5637	AGCUGGGCGCGGUGGCUCA	314	5655	UGAGCCACCGCGCCCAGCU	781
5655	AUGCCUGUAAUCCCAGCAC	315	5655	AUGCCUGUAAUCCCAGCAC	315	5673	GUGCUGGGAUUACAGGCAU	782
5673	CUUUGGGAGGCCGAGGCGG	316	5673	CUUUGGGAGGCCGAGGCGG	316	5691	CCGCCUCGGCCUCCCAAAG	783
5691	GGCAGAUCACCUGAGGUCG	317	5691	GGCAGAUCACCUGAGGUCG	317	5709	CGACCUCAGGUGAUCUGCC	784
5709	GGGAGGUCGAGACCAGCCU	318	5709	GGGAGGUCGAGACCAGCCU	318	5727	AGGCUGGUCUCGACCUCCC	785
5727	UGACCAACAUGGAGAAACC	319	5727	UGACCAACAUGGAGAAACC	319	5745	GGUUUCUCCAUGUUGGUCA	786
5745	CCCGUCUCUACUAAAAAUA	320	5745	CCCGUCUCUACUAAAAAUA	320	5763	UAUUUUUAGUAGAGACGGG	787
5763	ACAAAAUUAGCUGGGCGUG	321	5763	ACAAAAUUAGCUGGGCGUG	321	5781	CACGCCCAGCUAAUUUUGU	788
5781	GGUGGCUCAUGCCUGUAAU	322	5781	GGUGGCUCAUGCCUGUAAU	322	5799	AUUACAGGCAUGAGCCACC	789
5799	UCCCAGCUACUUGGGAGGC	323	5799	UCCCAGCUACUUGGGAGGC	323	5817	GCCUCCCAAGUAGCUGGGA	790
5817	CUGAGGCAGGAGAAUCGCU	324	5817	CUGAGGCAGGAGAAUCGCU	324	5835	AGCGAUUCUCCUGCCUCAG	791

5835	UUGAACCCAGGAGGCGGAG	325	5835	UUGAACCCAGGAGGCGGAG	325	5853	CUCCGCCUCCUGGGUUCAA	792
5853	GGUUGUGGUGAGCGAAGAU	326	5853	GGUUGUGGUGAGCGAAGAU	326	5871	AUCUUCGCUCACCACAACC	793
5871	UCGUGCCAUUGCACUCCAG	327	5871	UCGUGCCAUUGCACUCCAG	327	5889	CUGGAGUGCAAUGGCACGA	794
5889	GCCUGGGCAACAAGAGCAA	328	5889	GCCUGGGCAACAAGAGCAA	328	5907	UUGCUCUUGUUGCCCAGGC	795
5907	AAACUCUGUCUCAAAAAAA	329	5907	AAACUCUGUCUCAAAAAAA	329	5925	UUUUUUUGAGACAGAGUUU	796
5925	AAAAAAAGAUAUAAAUCAC	330	5925	AAAAAAAGAUAUAAAUCAC	330	5943	GUGAUUUAUAUCUUUUUUU	797
5943	CAAUAAAUAAAUAGGUCAA	331	5943	CAAUAAAUAAAUAGGUCAA	331	5961	UUGACCUAUUUAUUUAUUG	798
5961	AUACAAAUGUUAGCCAGGC	332	5961	AUACAAAUGUUAGCCAGGC	332	5979	GCCUGGCUAACAUUUGUAU	799
5979	CGUGGUGGCACAUGCCCAU	333	5979	CGUGGUGGCACAUGCCCAU	333	5997	AUGGGCAUGUGCCACCACG	800
5997	UAGUCGCAGCUACUCUGGA	334	5997	UAGUCGCAGCUACUCUGGA	334	6015	UCCAGAGUAGCUGCGACUA	801
6015	AGGCAGAGGCAGGAGGAUC	335	6015	AGGCAGAGGCAGGAGGAUC	335	6033	GAUCCUCCUGCCUCUGCCU	802
6033	CACUUGAGCCCAUGAAUUU	336	6033	CACUUGAGCCCAUGAAUUU	336	6051	AAAUUCAUGGGCUCAAGUG	803
6051	UGAGGCAGCAGUGAGCUAU	337	6051	UGAGGCAGCAGUGAGCUAU	337	6069	AUAGCUCACUGCUGCCUCA	804
6069	UGAUUGUGCCACUGUACUC	338	6069	UGAUUGUGCCACUGUACUC	338	6087	GAGUACAGUGGCACAAUCA	805
6087	CCAGUCUGGGUGACAGAGU	339	6087	CCAGUCUGGGUGACAGAGU	339	6105	ACUCUGUCACCCAGACUGG	806
6105	UGAGACCCCAUCUCUAAAU	340	6105	UGAGACCCCAUCUCUAAAU	340	6123	AUUUAGAGAUGGGGUCUCA	807
6123	UAAAUAGGUCAAACCCUUA	341	6123	UAAAUAGGUCAAACCCUUA	341	6141	UAAGGGUUUGACCUAUUUA	808
6141	AAAAAUAUUUAAAUUCUUA	342	6141	AAAAAUAUUUAAAUUCUUA	342	6159	UAAGAAUUUAAAUAUUUUU	809
6159	AAAAAAUUGAAAAGAUUAU	343	6159	AAAAAAUUGAAAAGAUUAU	343	6177	AUAAUCUUUUCAAUUUUUU	810
6177	UUCUUCUCAAAUUUAGUUG	344	6177	UUCUUCUCAAAUUUAGUUG	344	6195	CAACUAAAUUUGAGAAGAA	811
6195	GAGCUUUCUAAGAGAAGCA	345	6195	GAGCUUUCUAAGAGAAGCA	345	6213	UGCUUCUCUUAGAAAGCUC	812
6213	AAUUGGCUUUUUCCCACUU	346	6213	AAUUGGCUUUUUCCCACUU	346	6231	AAGUGGGAAAAAGCCAAUU	813
6231	UCAAUAAUCAUUUUCAGUU	347	6231	UCAAUAAUCAUUUUCAGUU	347	6249	AACUGAAAAUGAUUAUUGA	814
6249	UUGACUCAUACAGUUAACA	348	6249	UUGACUCAUACAGUUAACA	348	6267	UGUUAACUGUAUGAGUCAA	815
6267	ACAAUGUGAAUUUCUUCCU	349	6267	ACAAUGUGAAUUUCUUCCU	349	6285	AGGAAGAAAUUCACAUUGU	816
6285	UCAGCAUAACAGAGUUAUA	350	6285	UCAGCAUAACAGAGUUAUA	350	6303	UAUAACUCUGUUAUGCUGA	817
6303	AGAAUGACAGGGCUGGAAG	351	6303	AGAAUGACAGGGCUGGAAG	351	6321	CUUCCAGCCCUGUCAUUCU	818
6321	GUGACCUUAGAGAGUAUCC	352	6321	GUGACCUUAGAGAGUAUCC	352	6339	GGAUACUCUCUAAGGUCAC	819
6339	CAGUUCUUUCAUUUUACAG	353	6339	CAGUUCUUUCAUUUUACAG	353	6357	CUGUAAAAUGAAAGAACUG	820
6357	GGUGAGGCAACUGAGACUC	354	6357	GGUGAGGCAACUGAGACUC	354	6375	GAGUCUCAGUUGCCUCACC	821
6375	CAAAGGUGAUGUAAUUUGU	355	6375	CAAAGGUGAUGUAAUUUGU	355	6393	ACAAAUUACAUCACCUUUG	822
6393	UGCAAAGAUUAUAGCUAAU	356	6393	UGCAAAGAUUAUAGCUAAU	356	6411	AUUAGCUAUAAUCUUUGCA	823
6411	UUAGUAGCAGAGCCCUGAC	357	6411	UUAGUAGCAGAGCCCUGAC	357	6429	GUCAGGGCUCUGCUACUAA	824

6429	CUGGGACAUAGUUUGAAGG	358	6429	CUGGGACAUAGUUUGAAGG	358	6447	CCUUCAAACUAUGUCCCAG	825
6447	GUGAAAAACUUCACCAAGC	359	6447	GUGAAAAACUUCACCAAGC	359	6465	GCUUGGUGAAGUUUUUCAC	826
6465	CUACCUUUCUUGAAAGGUC	360	6465	CUACCUUUCUUGAAAGGUC	360	6483	GACCUUUCAAGAAAGGUAG	827
6483	CCAAAUGUUUAUGUUUUCA	361	6483	CCAAAUGUUUAUGUUUUCA	361	6501	UGAAAACAUAAACAUUUGG	828
6501	AACUACUCUUUCCACUGUA	362	6501	AACUACUCUUUCCACUGUA	362	6519	UACAGUGGAAAGAGUAGUU	829
6519	ACCAUAACUUUCACUACAU	363	6519	ACCAUAACUUUCACUACAU	363	6537	AUGUAGUGAAAGUUAUGGU	830
6537	UAUUAAAUGACACUUUAUA	364	6537	UAUUAAAUGACACUUUAUA	364	6555	UAUAAAGUGUCAUUUAAUA	831
6555	AACUAAUAUAAUAGGACAA	365	6555	AACUAAUAUAAUAGGACAA	365	6573	UUGUCCUAUUAUAUUAGUU	832
6573	AUCAUCAAUGCAUAUAUAG	366	6573	AUCAUCAAUGCAUAUAUAG	366	6591	CUAUAUAUGCAUUGAUGAU	833
6591	GCCAGCCCUUCAUAUCUGU	367	6591	GCCAGCCCUUCAUAUCUGU	367	6609	ACAGAUAUGAAGGGCUGGC	834
6609	UGGGUUUUGCAUCCAUGGA	368	6609	UGGGUUUUGCAUCCAUGGA	368	6627	UCCAUGGAUGCAAAACCCA	835
6627	AUUCAACCAAGGAGGAAUU	369	6627	AUUCAACCAAGGAGGAAUU	369	6645	AAUUCCUCCUUGGUUGAAU	836
6645	UGAAAACACUGAGAAAAAA	370	6645	UGAAAACACUGAGAAAAAA	370	6663	UUUUUUCUCAGUGUUUUCA	837
6663	AAAAAAAGACCACACAAUA	371	6663	AAAAAAAGACCACACAAUA	371	6681	UAUUGUGUGGUCUUUUUUU	838
6681	AAAAAAAAAAAAUACAAAA	372	6681	AAAAAAAAAAAAUACAAAA	372	6699	UUUUGUAUUUUUUUUUUUU	839
6699	AUAAUACAAAGAAAAAGCC	373	6699	AUAAUACAAAGAAAAAGCC	373	6717	GGCUUUUUCUUUGUAUUAU	840
6717	CAAAAUUGUCAUACUGUUG	374	6717	CAAAAUUGUCAUACUGUUG	374	6735	CAACAGUAUGACAAUUUUG	841
6735	GUUAAGCAACAGUAUAACA	375	6735	GUUAAGCAACAGUAUAACA	375	6753	UGUUAUACUGUUGCUUAAC	842
6753	AACUAUUUACAUAGCAUUA	376	6753	AACUAUUUACAUAGCAUUA	376	6771	UAAUGCUAUGUAAAUAGUU	843
6771	AAGGUUGGUGCAAAAAUGC	377	6771	AAGGUUGGUGCAAAAAUGC	377	6789	GCAUUUUUGCACCAACCUU	844
6789	CAAAAAAAAAAAAAGCAAU	378	6789	CAAAAAAAAAAAAAGCAAU	378	6807	AUUGCUUUUUUUUUUUUUG	845
6807	UUAUUUUUAAACCAACCUA	379	6807	UUAUUUUUAAACCAACCUA	379	6825	UAGGUUGGUUUAAAAAUAA	846
6825	AAUAUAUUGUAUUAGGUAU	380	6825	AAUAUAUUGUAUUAGGUAU	380	6843	AUACCUAAUACAAUAUAUU	847
6843	UUAAAGUCAUCUGGACAUG	381	6843	UUAAAGUCAUCUGGACAUG	381	6861	CAUGUCCAGAUGACUUUAA	848
6861	GAAUUAAAGUAUAUGAUGC	382	6861	GAAUUAAAGUAUAUGAUGC	382	6879	GCAUCAUAUACUUUAAUUC	849
6879	CCAGCCUGGACAAAAGGCA	383	6879	CCAGCCUGGACAAAAGGCA	383	6897	UGCCUUUUGUCCAGGCUGG	850
6897	AAAACCCUGUCUCUACAAA	384	6897	AAAACCCUGUCUCUACAAA	384	6915	UUUGUAGAGACAGGGUUUU	851
6915	AAAAUACAAAAAUUAGCUG	385	6915	AAAAUACAAAAAUUAGCUG	385	6933	CAGCUAAUUUUUGUAUUUU	852
6933	GGGCAUGGUGGUGUGUGCC	386	6933	GGGCAUGGUGGUGUGUGCC	386	6951	GGCACACACCACCAUGCCC	853
6951	CUGUAGUCCUGGCUACUCC	387	6951	CUGUAGUCCUGGCUACUCC	387	6969	GGAGUAGCCAGGACUACAG	854
6969	CGGAGCCUGAGGUGGGAGG	388	6969	CGGAGCCUGAGGUGGGAGG	388	6987	CCUCCCACCUCAGGCUCCG	855
6987	GAUCGCUUGAGUCUGGGAG	389	6987	GAUCGCUUGAGUCUGGGAG	389	7005	CUCCCAGACUCAAGCGAUC	856
7005	GGCAGAGGCUGCAUUGAGC	390	7005	GGCAGAGGCUGCAUUGAGC	390	7023	GCUCAAUGCAGCCUCUGCC	857

7023	CUAUGAUCAUGGCACUGCA	391	7023	CUAUGAUCAUGGCACUGCA	391	7041	UGCAGUGCCAUGAUCAUAG	858
7041	AUUCCAGCCUGGGUGACAG	392	7041	AUUCCAGCCUGGGUGACAG	392	7059	CUGUCACCCAGGCUGGAAU	859
7059	GUGCAAGACCUUGUCUCAG	393	7059	GUGCAAGACCUUGUCUCAG	393	7077	CUGAGACAAGGUCUUGCAC	860
7077	GAAUAAAUAAAGUAUGUGA	394	7077	GAAUAAAUAAAGUAUGUGA	394	7095	UCACAUACUUUAUUUAUUC	861
7095	AUGAAGAUGUGCAUACAUU	395	7095	AUGAAGAUGUGCAUACAUU	395	7113	AAUGUAUGCACAUCUUCAU	862
7113	UAUAUGCAAAUACUGUUUU	396	7113	UAUAUGCAAAUACUGUUUU	396	7131	AAAACAGUAUUUUGCAUAUA	863
7131	UUUUUUUUUUUAAUUUAAA	397	7131	UUUUUUUUUUUAAUUUAAA	397	7149	UUUAAAUUAAAAAAAAAAA	864
7149	ACAGUCUCACUGUGUUGCC	398	7149	ACAGUCUCACUGUGUUGCC	398	7167	GGCAACACAGUGAGACUGU	865
7167	CCAGGAUGGAGUGCAAUGG	399	7167	CCAGGAUGGAGUGCAAUGG	399	7185	CCAUUGCACUCCAUCCUGG	866
7185	GCACAAUCUUGGCUCAUGG	400	7185	GCACAAUCUUGGCUCAUGG	400	7203	CCAUGAGCCAAGAUUGUGC	867
7203	GCAAACUCUGCCUCGCAAG	401	7203	GCAAACUCUGCCUCGCAAG	401	7221	CUUGCGAGGCAGAGUUUGC	868
7221	GCAGCUGGGACUACAGGCA	402	7221	GCAGCUGGGACUACAGGCA	402	7239	UGCCUGUAGUCCCAGCUGC	869
7239	AUGCUCCACGGUGCCCAGU	403	7239	AUGCUCCACGGUGCCCAGU	403	7257	ACUGGGCACCGUGGAGCAU	870
7257	UUAAUUUUUUUUGUAUUCU	404	7257	UUAAUUUUUUUUGUAUUCU	404	7275	AGAAUACAAAAAAAUUAA	871
7275	UUAGUAGAGACAGGGUUUC	405	7275	UUAGUAGAGACAGGGUUUC	405	7293	GAAACCCUGUCUCUACUAA	872
7293	CACCAUGUUGGCCAGGCUA	406	7293	CACCAUGUUGGCCAGGCUA	406	7311	UAGCCUGGCCAACAUGGUG	873
7311	AGUCUUGAAUUUCUGACCU	407	7311	AGUCUUGAAUUUCUGACCU	407	7329	AGGUCAGAAAUUCAAGACU	874
7329	UCAAGUGAUUCAUCUCCCA	408	7329	UCAAGUGAUUCAUCUCCCA	408	7347	UGGGAGAUGAAUCACUUGA	875
7347	AAAGUGCUGGGAUUACAGG	409	7347	AAAGUGCUGGGAUUACAGG	409	7365	CCUGUAAUCCCAGCACUUU	876
7365	GCGUGAGCCACCACGGCCG	410	7365	GCGUGAGCCACCACGGCCG	410	7383	CGGCCGUGGUGGCUCACGC	877
7383	GGCUAAUUUUUGUAUUUUU	411	7383	GGCUAAUUUUUGUAUUUUU	411	7401	AAAAAUACAAAAAAUUAGCC	878
7401	UUAGUAGUGACUGGUUUCG	412	7401	UUAGUAGUGACUGGUUUCG	412	7419	CGAAACCAGUCACUACUAA	879
7419	GCGGUGUUGACCAGGCUGG	413	7419	GCGGUGUUGACCAGGCUGG	413	7437	CCAGCCUGGUCAACACCGC	880
7437	GUCUCGAACUCCUGAUCUC	414	7437	GUCUCGAACUCCUGAUCUC	414	7455	GAGAUCAGGAGUUUCGAGAC	881
7455	CAGGUGAUCUGCCUGCCUC	415	7455	CAGGUGAUCUGCCUGCCUC	415	7473	GAGGCAGGCAGAUCACCUG	882
7473	CGGCCUCACAAAGUGCUGG	416	7473	CGGCCUCACAAAGUGCUGG	416	7491	CCAGCACUUUGUGAGGCCG	883
7491	GGAUUACAGGUGUGAACCA	417	7491	GGAUUACAGGUGUGAACCA	417	7509	UGGUUCACACCUGUAAUCC	884
7509	ACUGCUCCCGGCCUUGUGU	418	7509	ACUGCUCCCGGCCUUGUGU	418	7527	ACACAAGGCCGGGAGCAGU	885
7527	UGAUUUUAUCUAAGGGACU	419	7527	UGAUUUUAUCUAAGGGACU	419	7545	AGUCCCUUAGAUAAAAUCA	886
7545	UUAAGCGUCCUCAGGUCCU	420	7545	UUAAGCGUCCUCAGGUCCU	420	7563	AGGACCUGAGGACGCUUAA	887
7563	UAGGGGGUCGUGAAACCAA	421	7563	UAGGGGGUCGUGAAACCAA	421	7581	UUGGUUUCACGACCCCCUA	888
7581	AAACCCCAGGGAUAGCAAG	422	7581	AAACCCCAGGGAUAGCAAG	422	7599	CUUGCUAUCCCUGGGGUUU	889
7599	GGGACAAUUGUAUCUUCAA	423	7599	GGGACAAUUGUAUCUUCAA	423	7617	UUGAAGAUACAAUUGUCCC	890

7617	AAGUAGACAAAUGGCGCCG	424	7617	AAGUAGACAAAUGGCGCCG	424	7635	CGGCGCCAUUUGUCUACUU	891
7635	GGGCACGGUGGCUCACGCC	425	7635	GGGCACGGUGGCUCACGCC	425	7653	GGCGUGAGCCACCGUGCCC	892
7653	CUGUAAUCCCAGCAGUUUC	426	7653	CUGUAAUCCCAGCAGUUUC	426	7671	GAAACUGCUGGGAUUACAG	893
7671	CCGAGGCUGAGGCAGGCGG	427	7671	CCGAGGCUGAGGCAGGCGG	427	7689	CCGCCUGCCUCAGCCUCGG	894
7689	GCUCACCUGAGGUCAGGAG	428	7689	GCUCACCUGAGGUCAGGAG	428	7707	CUCCUGACCUCAGGUGAGC	895
7707	GUUGGAGACCAGCCUGGCC	429	7707	GUUGGAGACCAGCCUGGCC	429	7725	GGCCAGGCUGGUCUCCAAC	896
7725	CAACAUGCUGAAACCCUGU	430	7725	CAACAUGCUGAAACCCUGU	430	7743	ACAGGGUUUCAGCAUGUUG	897
7743	UCUGUACAAAAAUACAAAA	431	7743	UCUGUACAAAAAUACAAAA	431	7761	UUUUGUAUUUUUGUACAGA	898
7761	AAUAGCUGGGCAUGGUGGC	432	7761	AAUAGCUGGGCAUGGUGGC	432	7779	GCCACCAUGCCCAGCUAUU	899
7779	CGCAUGCCUGUAGUCCCAG	433	7779	CGCAUGCCUGUAGUCCCAG	433	7797	CUGGGACUACAGGCAUGCG	900
7797	GCUACUAGAGCCGACUGAGG	434	7797	GCUACUAGAGCCGACUGAGG	434	7815	CCUCAGUCGCUCUAGUAGC	901
7815	GCAGGAGAAUUGCUUGAAC	435	7815	GCAGGAGAAUUGCUUGAAC	435	7833	GUUCAAGCAAUUCUCCUGC	902
7833	CCUGGGAGGCGGAGGUUGC	436	7833	CCUGGGAGGCGGAGGUUGC	436	7851	GCAACCUCCGCCUCCCAGG	903
7851	CAGGGAGCCAAGAUGGCGC	437	7851	CAGGGAGCCAAGAUGGCGC	437	7869	GCGCCAUCUUGGCUCCCUG	904
7869	CCACCGCACUCCAGCCUAG	438	7869	CCACCGCACUCCAGCCUAG	438	7887	CUAGGCUGGAGUGCGGUGG	905
7887	GGUGAUAGAGUGAGACUCC	439	7887	GGUGAUAGAGUGAGACUCC	439	7905	GGAGUCUCACUCUAUCACC	906
7905	CCUCUCAAAAACAAAACAA	440	7905	CCUCUCAAAAACAAAACAA	440	7923	UUGUUUUGUUUUUUGAGAGG	907
7923	AAACAAAAAAAUUAGACAA	441	7923	AAACAAAAAAAUUAGACAA	441	7941	UUGUCUAAUUUUUUUGUUU	908
7941	AAUGCUACAUUAAUGUUUG	442	7941	AAUGCUACAUUAAUGUUUG	442	7959	CAAACAUUAAUGUAGCAUU	909
7959	GGGUGGUCAGAUUCUACUU	443	7959	GGGUGGUCAGAUUCUACUU	443	7977	AAGUAGAAUCUGACCACCC	910
7977	UUGAAUCUGAAGUUUGCAG	444	7977	UUGAAUCUGAAGUUUGCAG	444	7995	CUGCAAACUUCAGAUUCAA	911
7995	GAUAUGCCUAUAGAUUUUU	445	7995	GAUAUGCCUAUAGAUUUUU	445	8013	AAAAAUCUAUAGGCAUAUC	912
8013	UGGAGUUUACCACUUUCUU	446	8013	UGGAGUUUACCACUUUCUU	446	8031	AAGAAAGUGGUAAAACUCCA	913
8031	UAUUCUGUAUCAUUAAUGU	447	8031	UAUUCUGUAUCAUUAAUGU	447	8049	ACAUUAAUGAUACAGAAUA	914
8049	UAAUAUUUAAAUUACUAU	448	8049	UAAUAUUUUAAAUUACUAU	448	8067	AUAGUAAUUUAAAAUAUUA	915
8067	UAUAUGUUACCAUUUUUCU	449	8067	UAUAUGUUACCAUUUUUCU	449	8085	AGAAAAAUGGUAACAUAUA	916
8085	UGGAUUUAGUAAGAAAUUU	450	8085	UGGAUUUAGUAAGAAAUUU	450	8103	AAAUUUCUUACUAAAUCCA	917
8103	UGCAGUUUUUGGUUUGAUGU	451	8103	UGCAGUUUUUGGUUUGAUGU	451	8121	ACAUCAAACCAAAACUGCA	918
8121	UAACAAGGGUUUUAAUGUA	452	8121	UAACAAGGGUUUUAAUGUA	452	8139	UACAUUAAAACCCUUGUUA	919
8139	AAUUUAUGUUAGAUUUUGC	453	8139	AAUUUAUGUUAGAUUUUGC	453	8157	GCAAAAUCUAACAUAAAUU	920
8157	CAUUUUUUUCAUUACUGUU	454	8157	CAUUUUUUUCAUUACUGUU	454	8175	AACAGUAAUGAAAAAAAUG	921
8175	UAUAUUUUAACCUGACUGA	455	8175	UAUAUUUUAACCUGACUGA	455	8193	UCAGUCAGGUUAAAAUAUA	922
8193	ACUGAUCUAAUUGUAUUAG	456	8193	ACUGAUCUAAUUGUAUUAG	456	8211	CUAAUACAAUUAGAUCAGU	923

8211	GUAUUGUGAAUAAUCAUGU	457	8211	GUAUUGUGAAUAAUCAUGU	457	8229	ACAUGAUUAUUCACAAUAC	924
8229	UGAAAUGUUUUGAGACAGA	458	8229	UGAAAUGUUUUGAGACAGA	458	8247	UCUGUCUCAAAACAUUUCA	925
8247	AGUACUAUAUUUGUGAAUA	459	8247	AGUACUAUAUUUGUGAAUA	459	8265	UAUUCACAAAUAUAGUACU	926
8265	AUAAUUUUAUGGUUUUUUU	460	8265	AUAAUUUUAUGGUUUUUUU	460	8283	AAAAAAACCAUAAAAUUAU	927
8283	UCACUUAGAACCUUUCUGU	461	8283	UCACUUAGAACCUUUCUGU	461	8301	ACAGAAAGGUUCUAAGUGA	928
8301	UGUGGAAAACUAAGAAAAU	462	8301	UGUGGAAAACUAAGAAAAU	462	8319	AUUUUCUUAGUUUUCCACA	929
8319	UUGCUUUCUGCUGUAUAAU	463	8319	UUGCUUUCUGCUGUAUAAU	463	8337	AUUAUACAGCAGAAAGCAA	930
8337	UCUGGCAUUCAUUGUAGAU	464	8337	UCUGGCAUUCAUUGUAGAU	464	8355	AUCUACAAUGAAUGCCAGA	931
8355	UUAAAGCUUAUUUUUCUGU	465	8355	UUAAAGCUUAUUUUUCUGU	465	8373	ACAGAAAAAUAAGCUUUAA	932
8373	UGAAUAAAACGUAUUCAAU	466	8373	UGAAUAAAACGUAUUCAAU	466	8391	AUUGAAUACGUUUUAUUCA	933
8391	UAAAAUACUAUUCUUUAAA	467	8391	UAAAAUACUAUUCUUUAAA	467	8409	UUUAAAGAAUAGUAUUUUA	934

The 3'-ends of the Upper sequence and the Lower sequence of the siNA construct can include an overhang sequence, for example about 1, 2, 3, or 4 nucleotides in length, preferably 2 nucleotides in length, wherein the overhanging sequence of the lower sequence is optionally complementary to a portion of the target sequence. The upper and lower sequences in the Table can further comprise a chemical modification having Formulae I-VII, such as exemplary siNA constructs shown in Figures 4 and 5, or having modifications described in Table IV or any combination thereof..

TABLE III: XIAP/BIRC4 Synthetic Modified siNA Constructs

Target Pos	Target	Seq ID	Cmpd#	Aliases	Sequence	Seq ID
94	GCGAAAAGGUGGACAAGUCCUAU	935		BIRC4:96U21 sense siNA	GAAAAGGUGGACAAGUCCUTT	943
314	GUGCUUUAGUUGUCAUGCAGCUG	936		BIRC4:316U21 sense siNA	GCUUUAGUUGUCAUGCAGCTT	944
353	UGGAGACUCAGCAGUUGGAAGAC	937		BIRC4:355U21 sense siNA	GAGACUCAGCAGUUGGAAGTT	945
358	ACUCAGCAGUUGGAAGACACAGG	938		BIRC4:360U21 sense siNA	UCAGCAGUUGGAAGACACATT	946
429	AGUGCCACGCAGUCUACAAAUUC	939		BIRC4:431U21 sense siNA	UGCCACGCAGUCUACAAAUTT	947
592	CGAGGAACCCUGCCAUGUAUAGU	940		BIRC4:594U21 sense siNA	AGGAACCCUGCCAUGUAUATT	948
667	CCCCAAGAGAGUUAGCAAGUGCU	941		BIRC4:669U21 sense siNA	CCAAGAGAGUUAGCAAGUGTT	949
1345	CACUUGAGGUUCUGGUUGCAGAU	942		BIRC4:1347U21 sense siNA	CUUGAGGUUCUGGUUGCAGTT	950
94	GCGAAAAGGUGGACAAGUCCUAU	935		BIRC4:114L21 antisense siNA (96C)	AGGACUUGUCCACCUUUUCTT	951
314	GUGCUUUAGUUGUCAUGCAGCUG	936		BIRC4:334L21 antisense siNA (316C)	GCUGCAUGACAACUAAAGCTT	952
353	UGGAGACUCAGCAGUUGGAAGAC	937		BIRC4:373L21 antisense siNA (355C)	CUUCCAACUGCUGAGUCUCTT	953
358	ACUCAGCAGUUGGAAGACACAGG	938		BIRC4:378L21 antisense siNA (360C)	UGUGUCUUCCAACUGCUGATT	954
429	AGUGCCACGCAGUCUACAAAUUC	939		BIRC4:449L21 antisense siNA (431C)	AUUUGUAGACUGCGUGGCATT	955
592	CGAGGAACCCUGCCAUGUAUAGU	940		BIRC4:612L21 antisense siNA (594C)	UAUACAUGGCAGGGUUCCUTT	956
667	CCCCAAGAGAGUUAGCAAGUGCU	941		BIRC4:687L21 antisense siNA (669C)	CACUUGCUAACUCUCUUGGTT	957
1345	CACUUGAGGUUCUGGUUGCAGAU	942		BIRC4:1365L21 antisense siNA (1347C)	CUGCAACCAGAACCUCAAGTT	958
94	GCGAAAAGGUGGACAAGUCCUAU	935		BIRC4:96U21 sense siNA stab04	B GAAAAGGuGGAcAAGuccuTT B	959
314	GUGCUUUAGUUGUCAUGCAGCUG	936		BIRC4:316U21 sense siNA stab04	B GcuuuAGuuGucAuGcAGcTT B	960
353	UGGAGACUCAGCAGUUGGAAGAC	937		BIRC4:355U21 sense siNA stab04	B GAGAcucAGcAGuuGGAAGTT B	961
358	ACUCAGCAGUUGGAAGACACAGG	938		BIRC4:360U21 sense siNA stab04	B ucAGcAGuuGGAAGAcAcATT B	962
429	AGUGCCACGCAGUCUACAAAUUC	939		BIRC4:431U21 sense siNA stab04	B uGccAcGcAGucuAcAAAuTT B	963
592	CGAGGAACCCUGCCAUGUAUAGU	940		BIRC4:594U21 sense siNA stab04	B AGGAAcccuGccAuGuAuATT B	964
667	CCCCAAGAGAGUUAGCAAGUGCU	941		BIRC4:669U21 sense siNA stab04	B ccAAGAGAGuuAGcAAGuGTT B	965
1345	CACUUGAGGUUCUGGUUGCAGAU	942		BIRC4:1347U21 sense siNA stab04	B cuuGAGGuucuGGuuGcAGTT B	966
94	GCGAAAAGGUGGACAAGUCCUAU	935		BIRC4:114L21 antisense siNA (96C) stab05	AGGAcuuGuccAccuuuucTsT	967
314	GUGCUUUAGUUGUCAUGCAGCUG	936		BIRC4:334L21 antisense siNA (316C) stab05	GcuGcAuGAcAAcuAAAGcTsT	968
353	UGGAGACUCAGCAGUUGGAAGAC	937		BIRC4:373L21 antisense siNA (355C) stab05	cuuccAAcuGcuGAGucucTsT	969
358	ACUCAGCAGUUGGAAGACACAGG	938		BIRC4:378L21 antisense siNA (360C) stab05	uGuGucuuccAAcuGcuGATsT	970

429	AGUGCCACGCAGUCUACAAAUUC	939		BIRC4:449L21 antisense siNA (431C) stab05	AuuuGuAGAcuGcGuGGcATsT	971
592	CGAGGAACCCUGCCAUGUAUAGU	940		BIRC4:612L21 antisense siNA (594C) stab05	uAuAcAuGGcAGGGuuccuTsT	972
667	CCCCAAGAGAGUUAGCAAGUGCU	941		BIRC4:687L21 antisense siNA (669C) stab05	cAcuuGcuAAcucucuuGGTsT	973
1345	CACUUGAGGUUCUGGUUGCAGAU	942		BIRC4:1365L21 antisense siNA (1347C) stab05	cuGcAAccAGAAccucAAGTsT	974
94	GCGAAAAGGUGGACAAGUCCUAU	935		BIRC4:96U21 sense siNA stab07	B GAAAAGGuGGAcAAGuccuTT B	975
314	GUGCUUUAGUUGUCAUGCAGCUG	936		BIRC4:316U21 sense siNA stab07	B GcuuuAGuuGucAuGcAGcTT B	976
353	UGGAGACUCAGCAGUUGGAAGAC	937		BIRC4:355U21 sense siNA stab07	B GAGAcucAGcAGuuGGAAGTT B	977
358	ACUCAGCAGUUGGAAGACACAGG	938		BIRC4:360U21 sense siNA stab07	B ucAGcAGuuGGAAGAcAcATT B	978
429	AGUGCCACGCAGUCUACAAAUUC	939		BIRC4:431U21 sense siNA stab07	B uGccAcGcAGucuAcAAAuTT B	979
592	CGAGGAACCCUGCCAUGUAUAGU	940		BIRC4:594U21 sense siNA stab07	B AGGAAcccuGccAuGuAuATT B	980
667	CCCCAAGAGAGUUAGCAAGUGCU	941		BIRC4:669U21 sense siNA stab07	B ccAAGAGAGuuAGcAAGuGTT B	981
1345	CACUUGAGGUUCUGGUUGCAGAU	942		BIRC4:1347U21 sense siNA stab07	B cuuGAGGuucuGGuuGcAGTT B	982
94	GCGAAAAGGUGGACAAGUCCUAU	935		BIRC4:114L21 antisense siNA (96C) stab11	AGGAcuuGuccAccuuuucTsT	983
314	GUGCUUUAGUUGUCAUGCAGCUG	936		BIRC4:334L21 antisense siNA (316C) stab11	GcuGcAuGAcAAcuAAAGcTsT	984
353	UGGAGACUCAGCAGUUGGAAGAC	937		BIRC4:373L21 antisense siNA (355C) stab11	cuuccAAcuGcuGAGucucTsT	985
358	ACUCAGCAGUUGGAAGACACAGG	938		BIRC4:378L21 antisense siNA (360C) stab11	uGuGucuuccAAcuGcuGATsT	986
429	AGUGCCACGCAGUCUACAAAUUC	939		BIRC4:449L21 antisense siNA (431C) stab11	AuuuGuAGAcuGcGuGGcATsT	987
592	CGAGGAACCCUGCCAUGUAUAGU	940		BIRC4:612L21 antisense siNA (594C) stab11	uAuAcAuGGcAGGGuuccuTsT	988
667	CCCCAAGAGAGUUAGCAAGUGCU	941		BIRC4:687L21 antisense siNA (669C) stab11	cAcuuGcuAAcucucuuGGTsT	989
1345	CACUUGAGGUUCUGGUUGCAGAU	942		BIRC4:1365L21 antisense siNA (1347C) stab11	cuGcAAccAGAAccucAAGTsT	990
94	GCGAAAAGGUGGACAAGUCCUAU	935		BIRC4:96U21 sense siNA stab18	B GAAAAGGuGGAcAAGuccuTT B	991
314	GUGCUUUAGUUGUCAUGCAGCUG	936		BIRC4:316U21 sense siNA stab18	B GcuuuAGuuGucAuGcAGcTT B	992
353	UGGAGACUCAGCAGUUGGAAGAC	937		BIRC4:355U21 sense siNA stab18	B GAGAcucAGcAGuuGGAAGTT B	993
358	ACUCAGCAGUUGGAAGACACAGG	938		BIRC4:360U21 sense siNA stab18	B ucAGcAGuuGGAAGAcAcATT B	994
429	AGUGCCACGCAGUCUACAAAUUC	939		BIRC4:431U21 sense siNA stab18	B uGccAcGcAGucuAcAAAuTT B	995
592	CGAGGAACCCUGCCAUGUAUAGU	940		BIRC4:594U21 sense siNA stab18	B AGGAAcccuGccAuGuAuATT B	996
667	CCCCAAGAGAGUUAGCAAGUGCU	941		BIRC4:669U21 sense siNA stab18	B ccAAGAGAGuuAGcAAGuGTT B	997
1345	CACUUGAGGUUCUGGUUGCAGAU	942		BIRC4:1347U21 sense siNA stab18	B cuuGAGGuucuGGuuGcAGTT B	998
94	GCGAAAAGGUGGACAAGUCCUAU	935	33645	BIRC4:114L21 antisense siNA (96C) stab08	AGGAcuuGuccAccuuuucTsT	999
314	GUGCUUUAGUUGUCAUGCAGCUG	936	33646	BIRC4:334L21 antisense siNA (316C) stab08	GcuGcAuGAcAAcuAAAGcTsT	1000
353	UGGAGACUCAGCAGUUGGAAGAC	937	33647	BIRC4:373L21 antisense siNA (355C) stab08	cuuccAAcuGcuGAGucucTsT	1001
358	ACUCAGCAGUUGGAAGACACAGG	938	33648	BIRC4:378L21 antisense siNA (360C) stab08	uGuGucuuccAAcuGcuGATsT	1002

429	AGUGCCACGCAGUCUACAAAUUC	939	33649	BIRC4:449L21 antisense siNA (431C) stab08	AuuuGuAGAcuGcGuGGcATsT	1003
592	CGAGGAACCCUGCCAUGUAUAGU	940	33650	BIRC4:612L21 antisense siNA (594C) stab08	uAuAcAuGGcAGGGuuccuTsT	1004
667	CCCCAAGAGAGUUAGCAAGUGCU	941	33651	BIRC4:687L21 antisense siNA (669C) stab08	cAcuuGcuAAcucucuuGGTsT	1005
1345	CACUUGAGGUUCUGGUUGCAGAU	942	33652	BIRC4:1365L21 antisense siNA (1347C) stab08	cuGcAAccAGAAccucAAGTsT	1006
94	GCGAAAAGGUGGACAAGUCCUAU	935	33629	BIRC4:96U21 sense siNA stab09	B GAAAAGGUGGACAAGUCCUTT B	1007
314	GUGCUUUAGUUGUCAUGCAGCUG	936	33630	BIRC4:316U21 sense siNA stab09	B GCUUUAGUUGUCAUGCAGCTT B	1008
353	UGGAGACUCAGCAGUUGGAAGAC	937	33631	BIRC4:355U21 sense siNA stab09	B GAGACUCAGCAGUUGGAAGTT B	1009
358	ACUCAGCAGUUGGAAGACACAGG	938	33632	BIRC4:360U21 sense siNA stab09	B UCAGCAGUUGGAAGACACATT B	1010
429	AGUGCCACGCAGUCUACAAAUUC	939	33633	BIRC4:431U21 sense siNA stab09	B UGCCACGCAGUCUACAAAUTT B	1011
592	CGAGGAACCCUGCCAUGUAUAGU	940	33634	BIRC4:594U21 sense siNA stab09	B AGGAACCCUGCCAUGUAUATT B	1012
667	CCCCAAGAGAGUUAGCAAGUGCU	941	33635	BIRC4:669U21 sense siNA stab09	B CCAAGAGAGUUAGCAAGUGTT B	1013
1345	CACUUGAGGUUCUGGUUGCAGAU	942	33636	BIRC4:1347U21 sense siNA stab09	B CUUGAGGUUCUGGUUGCAGTT B	1014
94	GCGAAAAGGUGGACAAGUCCUAU	935	33637	BIRC4:114L21 antisense siNA (96C) stab10	AGGACUUGUCCACCUUUUCTsT	1015
314	GUGCUUUAGUUGUCAUGCAGCUG	936	33638	BIRC4:334L21 antisense siNA (316C) stab10	GCUGCAUGACAACUAAAGCTsT	1016
353	UGGAGACUCAGCAGUUGGAAGAC	937	33639	BIRC4:373L21 antisense siNA (355C) stab10	CUUCCAACUGCUGAGUCUCTsT	1017
358	ACUCAGCAGUUGGAAGACACAGG	938	33640	BIRC4:378L21 antisense siNA (360C) stab10	UGUGUCUUCCAACUGCUGATsT	1018
429	AGUGCCACGCAGUCUACAAAUUC	939	33641	BIRC4:449L21 antisense siNA (431C) stab10	AUUUGUAGACUGCGUGGCATsT	1019
592	CGAGGAACCCUGCCAUGUAUAGU	940	33642	BIRC4:612L21 antisense siNA (594C) stab10	UAUACAUGGCAGGGUUCCUTsT	1020
667	CCCCAAGAGAGUUAGCAAGUGCU	941	33643	BIRC4:687L21 antisense siNA (669C) stab10	CACUUGCUAACUCUCUUGGTsT	1021
1345	CACUUGAGGUUCUGGUUGCAGAU	942	33644	BIRC4:1365L21 antisense siNA (1347C) stab10	CUGCAACCAGAACCUCAAGTsT	1022
94	GCGAAAAGGUGGACAAGUCCUAU	935		BIRC4:114L21 antisense siNA (96C) stab19	AGGAcuuGuccAccuuuucTT B	1023
314	GUGCUUUAGUUGUCAUGCAGCUG	936		BIRC4:334L21 antisense siNA (316C) stab19	GcuGcAuGAcAAcuAAAGcTT B	1024
353	UGGAGACUCAGCAGUUGGAAGAC	937		BIRC4:373L21 antisense siNA (355C) stab19	cuuccAAcuGcuGAGucucTT B	1025
358	ACUCAGCAGUUGGAAGACACAGG	938		BIRC4:378L21 antisense siNA (360C) stab19	uGuGucuuccAAcuGcuGATT B	1026
429	AGUGCCACGCAGUCUACAAAUUC	939		BIRC4:449L21 antisense siNA (431C) stab19	AuuuGuAGAcuGcGuGGcATT B	1027
592	CGAGGAACCCUGCCAUGUAUAGU	940		BIRC4:612L21 antisense siNA (594C) stab19	uAuAcAuGGcAGGGuuccuTT B	1028
667	CCCCAAGAGAGUUAGCAAGUGCU	941		BIRC4:687L21 antisense siNA (669C) stab19	cAcuuGcuAAcucucuuGGTT B	1029
1345	CACUUGAGGUUCUGGUUGCAGAU	942		BIRC4:1365L21 antisense siNA (1347C) stab19	cuGcAAccAGAAccucAAGTT B	1030
94	GCGAAAAGGUGGACAAGUCCUAU	935		BIRC4:114L21 antisense siNA (96C) stab22	AGGACUUGUCCACCUUUUCTT B	1031
314	GUGCUUUAGUUGUCAUGCAGCUG	936		BIRC4:334L21 antisense siNA (316C) stab22	GCUGCAUGACAACUAAAGCTT B	1032
353	UGGAGACUCAGCAGUUGGAAGAC	937		BIRC4:373L21 antisense siNA (355C) stab22	CUUCCAACUGCUGAGUCUCTT B	1033
358	ACUCAGCAGUUGGAAGACACAGG	938		BIRC4:378L21 antisense siNA (360C) stab22	UGUGUCUUCCAACUGCUGATT B	1034

429	AGUGCCACGCAGUCUACAAAUUC	939	BIRC4:449L21 antisense siNA (431C) stab22	AUUUGUAGACUGCGUGGCATT B	1035
592	CGAGGAACCCUGCCAUGUAUAGU	940	BIRC4:612L21 antisense siNA (594C) stab22	UAUACAUGGCAGGGUUCCUTT B	1036
667	CCCCAAGAGAGUUAGCAAGUGCU	941	BIRC4:687L21 antisense siNA (669C) stab22	CACUUGCUAACUCUCUUGGTT B	1037
1345	CACUUGAGGUUCUGGUUGCAGAU	942	BIRC4:1365L21 antisense siNA (1347C) stab22	CUGCAACCAGAACCUCAAGTT B	1038

Uppercase = ribonucleotide
u,c = 2'-deoxy-2'-fluoro U,C
T = thymidine
B = inverted deoxy abasic
s = phosphorothioate linkage

A = deoxy Adenosine
G = deoxy Guanosine
G = 2'-O-methyl Guanosine
A = 2'-O-methyl Adenosine

Table IV

Non-limiting examples of Stabilization Chemistries for chemically modified siNA constructs

Chemistry	pyrimidine	Purine	cap	p=S	Strand
"Stab 00"	Ribo	Ribo	TT at 3'-ends		S/AS
"Stab 1"	Ribo	Ribo	-	5 at 5'-end 1 at 3'-end	S/AS
"Stab 2"	Ribo	Ribo	-	All linkages	Usually AS
"Stab 3"	2'-fluoro	Ribo	-	4 at 5'-end 4 at 3'-end	Usually S
"Stab 4"	2'-fluoro	Ribo	5' and 3'-ends	-	Usually S
"Stab 5"	2'-fluoro	Ribo	-	1 at 3'-end	Usually AS
"Stab 6"	2'-O-Methyl	Ribo	5' and 3'-ends	-	Usually S
"Stab 7"	2'-fluoro	2'-deoxy	5' and 3'-ends	-	Usually S
"Stab 8"	2'-fluoro	2'-O-Methyl	-	1 at 3'-end	S/AS
"Stab 9"	Ribo	Ribo	5' and 3'-ends	-	Usually S
"Stab 10"	Ribo	Ribo	-	1 at 3'-end	Usually AS
"Stab 11"	2'-fluoro	2'-deoxy	-	1 at 3'-end	Usually AS
"Stab 12"	2'-fluoro	LNA	5' and 3'-ends		Usually S
"Stab 13"	2'-fluoro	LNA		1 at 3'-end	Usually AS
"Stab 14"	2'-fluoro	2'-deoxy		2 at 5'-end 1 at 3'-end	Usually AS
"Stab 15"	2'-deoxy	2'-deoxy		2 at 5'-end 1 at 3'-end	Usually AS
"Stab 16"	Ribo	2'-O-Methyl	5' and 3'-ends		Usually S
"Stab 17"	2'-O-Methyl	2'-O-Methyl	5' and 3'-ends		Usually S
"Stab 18"	2'-fluoro	2'-O-Methyl	5' and 3'-ends		Usually S
"Stab 19"	2'-fluoro	2'-O-Methyl	3'-end		S/AS
"Stab 20"	2'-fluoro	2'-deoxy	3'-end		Usually AS
"Stab 21"	2'-fluoro	Ribo	3'-end		Usually AS
"Stab 22"	Ribo	Ribo	3'-end		Usually AS
"Stab 23"	2'-fluoro*	2'-deoxy*	5' and 3'-ends		Usually S
"Stab 24"	2'-fluoro*	2'-O-Methyl*	-	1 at 3'-end	S/AS
"Stab 25"	2'-fluoro*	2'-O-Methyl*	-	1 at 3'-end	S/AS
"Stab 26"	2'-fluoro*	2'-O-	-		S/AS

		Methyl*			
"Stab 27"	2'-fluoro*	2'-O-Methyl*	3'-end		S/AS
"Stab 28"	2'-fluoro*	2'-O-Methyl*	3'-end		S/AS

CAP = any terminal cap, see for example **Figure 10.**
All Stab 00-28 chemistries can comprise 3'-terminal thymidine (TT) residues
All Stab 00-28 chemistries typically comprise about 21 nucleotides, but can vary as described herein.
S = sense strand
AS = antisense strand
*Stab 23 has a single ribonucleotide adjacent to 3'-CAP
*Stab 24 and Stab 28 have a single ribonucleotide at 5'-terminus
*Stab 25, Stab 26, and Stab 27 have three ribonucleotides at 5'-terminus
p = phosphorothioate linkage

Table V

A. 2.5 µmol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	6.5	163 µL	45 sec	2.5 min	7.5 min
S-Ethyl Tetrazole	23.8	238 µL	45 sec	2.5 min	7.5 min
Acetic Anhydride	100	233 µL	5 sec	5 sec	5 sec
N-Methyl Imidazole	186	233 µL	5 sec	5 sec	5 sec
TCA	176	2.3 mL	21 sec	21 sec	21 sec
Iodine	11.2	1.7 mL	45 sec	45 sec	45 sec
Beaucage	12.9	645 µL	100 sec	300 sec	300 sec
Acetonitrile	NA	6.67 mL	NA	NA	NA

B. 0.2 µmol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	15	31 µL	45 sec	233 sec	465 sec
S-Ethyl Tetrazole	38.7	31 µL	45 sec	233 min	465 sec
Acetic Anhydride	655	124 µL	5 sec	5 sec	5 sec
N-Methyl Imidazole	1245	124 µL	5 sec	5 sec	5 sec
TCA	700	732 µL	10 sec	10 sec	10 sec
Iodine	20.6	244 µL	15 sec	15 sec	15 sec
Beaucage	7.7	232 µL	100 sec	300 sec	300 sec
Acetonitrile	NA	2.64 mL	NA	NA	NA

C. 0.2 µmol Synthesis Cycle 96 well Instrument

Reagent	Equivalents:DNA/ 2'-O-methyl/Ribo	Amount: DNA/2'-O-methyl/Ribo	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time* Ribo
Phosphoramidites	22/33/66	40/60/120 µL	60 sec	180 sec	360sec
S-Ethyl Tetrazole	70/105/210	40/60/120 µL	60 sec	180 min	360 sec
Acetic Anhydride	265/265/265	50/50/50 µL	10 sec	10 sec	10 sec
N-Methyl Imidazole	502/502/502	50/50/50 µL	10 sec	10 sec	10 sec
TCA	238/475/475	250/500/500 µL	15 sec	15 sec	15 sec
Iodine	6.8/6.8/6.8	80/80/80 µL	30 sec	30 sec	30 sec
Beaucage	34/51/51	80/120/120	100 sec	200 sec	200 sec
Acetonitrile	NA	1150/1150/1150 µL	NA	NA	NA

- Wait time does not include contact time during delivery.
- Tandem synthesis utilizes double coupling of linker molecule